DIVERSITY OF BIOFOULING ORGANISMS ON OPEN SEA CAGES IN RELATION TO ENVIRONMENT

Synopsis submitted by

Sonali S. Mhaddolkar, M.Sc. M.Phil.

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
P.B.No.1603, Kochi, Kerala- 682018

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Under the guidance of
Dr. Dineshbabu A.P. Ph. D
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
P.B.No.1603, Kochi, Kerala- 682018

DEPARTMENT OF BIOSCIENCES

MANGALORE UNIVERSITY, MANGALGANGOTRI,

KARNATAKA, INDIA

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Chapter 1: Introduction

Worldwide, rising demands for quality sea food is a major cause of concern. Due to stagnation in commercial fish catches, fishing communities who merely depend on the capture fisheries resources for their livelihood are also facing uncertain future due to reduced catches. As per FAO estimates (FAO, 2014), India stands second in the aquaculture production among the five major aquaculture producing countries during 2014.

Global mariculture production (inclusive of coastal aquaculture) is 30.8 mt. in 2018 and among them fin fish production is 7.3mt. (FAO, 2020). Globally marine cage culture started long back as an additional source of marine fish supply but later the stagnation and the decline in marine fin fish stock forced most of the countries to initiate finfish culture as a mandatory fish production system (Shobika et al., 2020) to ensure the sustainable marine fish supply to the population, which was eventually accepted by Indian fisheries sector also.

Sea cage culture

Marine cage farming or sea cage culture is referred to culturing marine shell fishes and fin fishes in closed systems (cages) in the sea. In India, open sea cage culture of marine finfish and shellfish has been initiated in 2007, by ICAR-Central Marine Fisheries Research Institute, Govt. of India and succeeded in cage designing, culture and production of a number of marine finfish species in cages (Rao et al., 2013; Gopalakrishnan et al., 2019).

Mariculture

The economical farming of important marine organisms for different uses in enclosures (Phillips et al., 1985) in sea or onshore hatcheries, nursery and RAC systems is mariculture. In 1970, Central Salt and Marine Chemicals Research Institute initiated mariculture in India. In 1980s ICAR-CMFRI has initiated the mariculture activities and in 1990s small scale commercial activities in marine cage culture have been initiated (NMP, 2018).

Marine biofouling

Cage maintenance is an important aspect in open sea cage culture. Since the cage is installed in open sea, fouling will be a regular phenomenon. The unwanted growths of marine life forms on the submerged surfaces are referred as marine biofouling. The fishes are generally reared in nets of multiple layer, outer ones protection from outside intervention and the inner one provide protected living space for fishes. Marine biofouling is invariably associated with sea cage farming. Biofouling organisms are responsible for the degradation of cage nets and other structures causing serious problems in open sea cage farming. Biofoulers are composed of diverse group of organisms starting from the micro foulers like bacteria and diatoms to the macrofoulers like green mussels, oysters, barnacle etc. Biofouling is broadly divided into micro fouling and macro fouling.

Biofouling and accumulation of the fouling organisms has affected the finfish open sea cage culture and shell fish farming (Hopkins et al., 2021; Giangrande et al., 2020). It is essential to understand the biological cycle and ecological succession (Giangrande et al., 2020). So that better understanding can be obtained about foulers which can affect the performance of the cage nets by way of preventing flow of water into the cage and from the cage.

Information on biofoulers is one of the most important basic information in mariculture site selection. Information on biofouling intensity in space and time can be incorporated in cage site selection as decision making input and for the countries like India, where cage culture is in infancy phase and is developing fast, a detailed study report on this aspect is extremely essential. Attempt for spatio-temporal inventory of biofoulers and the research on biofouling organisms were not so far initiated from Indian waters. The present study aims to systematically inventorise the marine biofoulers, and to study the impact of biofouling on different structures or cage components, to ensure the feasibility in Indian mariculture industry.

Objectives of the study:

1. To study the diversity of fouling organism on open sea cage structures
2. To study the density of fouling community
3. To understand the seasonal variation in the fouling
4. To evaluate the biomass of fouling community
5. To understand the species composition of the fouling community and ecological climaxing on the cages
6. To understand the species succession among the fouling community
7. To study the influence of environmental parameters on the occurrence of fouling organisms

Study focus:

Study is focused to

- describe the composition, distribution, density, diversity, biomass, succession and correlation with the environmental parameters of biofouling organisms associated with fish cage farm in the West coast of India, in the Arabian Sea, off Karwar, Karnataka, India to ensure feasibility of cage farming in that area.
- estimate the density of macrofoulers between the three depths, and prominent foulers throughout the culture period and dominant fouling communities among the macro foulers, on the experimental net panels, cage nets and barrels.
- collect information on distribution of biofoulers is taken as criteria for selecting site for the culture.
- aim to inform the fish farmers about temporal occurrence of biofouling communities that cause economic loss during culture period.

Chapter 2: Review of literature

Production of marine finfish through cage culture practices has been increasing globally, and the culture is already well established in many Asian countries (DeSilva and Phillips et al., 2007; Sudirman et al., 2009). Open sea cage farming is viewed as a major opportunity for enhancing the marine food production and has been growing rapidly at global level. Open sea cage culture aspects in Indian waters such as site, cage structure, growth and culture were well documented recently (Joseph et al., 2010; Philippose et al., 2013; Rao et al., 2013).

Marine biofouling and study on biofoulers have been a topic of great interest worldwide, even before introduction of cage farming. Importance of understanding biofouling population in cage culture installations is an extension of these investigations and several researchers (Boyd, 1972; Bacchiocchi and Airoldi, 2003; Railkin, 2004; Pati et al., 2015) worked on this topic in great detail from different parts of the world. In recent review article, Hopkins et al. (2021) has described the importance of the investigations of marine biofouling on the marine SSAS (submerged-static-artificial-structures) for better management of biofoulers. Considering the biofouling as a menace, many technologies are being attempted to reduce biofouling in recent years (Ashraf et al., 2020; Hopkins et al., 2021).

In India, even though the present study on the impact of biofoulers in sea cages is a new attempt, researchers (Nagabhushanam, 1997; Nair et al., 1988; Desai and Prakash, 2009) provided baseline information on marine biofouling on fixed substrates. Many research works were carried on the fouling community structure (Ismail and Azariah, 1978; Rao and Balaji, 1994; Pati et al., 2015; Sahu et al., 2011). They conducted studies on different structures such as experimental panels, ship hulls, jetties etc. In India marine fouling was reported as major problem in pearl oyster culture farms (FAO, 2017). Since the open sea cage culture has been established in recent years (CMFRI, 2010, 2011, 2012, 2013), literature for biofouling studies on cage structure and cage accessories were meager and were limited to reports. But there is a lacuna in literature for biofoulers study

in finfish mariculture sites in India, leading to lot of opportunities in this field for investigations.

2.1 Biofoulers, composition and distribution:

According to estimates over 4,000 organisms, responsible for biofouling have been identified around the world (Cao et al., 2011; Bressy and Lejars, 2014). The intensity of fouling varied in tropical and temperate region, as well as coastal and open waters (Braithwaite and McEvoy, 2005; Bloecher et al., 2013) depending on the species diversity (Clements, 1936).

Many biofouling studies have been carried out in aquaculture cages in USA and Norway (Greene and Grizzle, 2007; Bloecher et al., 2013). About 34 marine fouling species have been found attached on the cage nets, which were immersed for about two months (Cheah and Chua, 1979). Recently, 117 taxa were documented from two farming sites in Mediterranean Sea (Giangrande et al., 2020). In East coast of India, 30 biofouling taxa were documented on the wooden panels in Kalpakkam jetty, 121 from Vishakhapatnam harbor, 37 from Kakinada. In the west coast, 65 taxa were recorded from Goa (Anil and Wagh, 1988; Sahu et al., 2011) but such documentations were based on biofouling on general hard structures, not on cage culture structures.

Biofouling community

Detailed investigations on fouling communities associated with aquaculture and their impacts in Malaysia, UK, Australia, USA, Singapore and Norway were reviewed (Fitridge et al., 2012). Comparative studies on development of biofouling communities of cage culture site and the non-caged (or reference site) were carried out in European waters (Cook et al., 2006). Biofouling is highly site specific (Sahu et al., 2011) and in India, general fouling community structure were a topic of study for number of scientists (Ismail and Azariah, 1978; Rao and Balaji, 1994; Pati et al., 2015 and Sahu, et al., 2011). But the concerns on biofouling communities related to sea cage culture are very recent in origin in India (CMFRI, 2010, 2011, 2012, 2013).

2.2 Monthly and seasonal variations in density of biofoulers:

Worldwide, many studies have been conducted on the biofoulers aggregation on the salmon culture infrastructure facilities (Floerl et al., 2016; Fitridge et al., 2012). There are

records of salmon farming areas where more than 60 percentages of culture net surfaces are covered by biofouers, forming total net occlusion during culture (Floerl et al., 2016; Braithwaite et al., 2007; Cronin et al., 1999). Literature is available on biofoulers abundance and density variation among various sites (Guenther et al., 2010; Fitridge et al., 2012; Cook et al., 2006).

2.3. Monthly and seasonal variations in biomass of biofoulers

Biofoulers add extra weight to equipments used for culture, resulting with increased culture cost (Fitridge et al., 2012). In the deeper water the biofouling community biomass decreases (Cronin et al., 1999; Guenther et al., 2010; Fitridge et al., 2012). The fouling biomass studies, community succession, in Norwegian cage culture are well studied (Bloecher et al., 2013; Braithwaite and McEvoy, 2005).

The biofoulers add extra weight on cage structure which eventually increase the production cost and maintenance cost (Pudota, 2011; Braithwaith and McEvoy, 2005; Guenther et al., 2010; deNys and Guenther, 2009)

2.4. Community succession of biofoulers

Clements theory (Clements, 1936) is an idealistic theory proposing climax as the final stage of succession process. Final succession pattern after immersion in two 'integrated multi trophic aquaculture (IMTA)' farms were documented (Giangrande et al., 2020). Ecological succession studies at different depth were carried out by Dziubinska and Szaniawska (2011). Literature on succession studies on biofoulers on marine cages of Gulf of Maine, United States of America (Greene and Grizzle, 2007) documented the succession of biofoulers. The ecological process of fouling communities development found to take place within a day or in weeks (Fitridge et al., 2012) and the composition of fouler communities varied both spatially and temporally (Fraschetti et al., 2005; Fitridge et al., 2012; Hollaway and Keough, 2002). In east coast of India, succession pattern on the wooden panels were studied in a Jetty at Kalpakkam waters (Sahu et al., 2011).

2.5 Diversity indices of biofoulers

The species diversity is the measure of relative abundance, and the diversity measurements are more widely used in the ecological community studies (Tokeshi, 1993). The fouling diversity studies, species composition, on the panels have been well

investigated in sea cage farming, mainly in the salmons culture (Bloecher et al., 2013; Braithwaite and McEvoy, 2005). Various researchers studied on early fouling like hydroid fouling studies in Norway and Germany (Kassah, 2012; Carl et al., 2011; Guenther et al., 2010; Sousa, 1979). Utility of information on diversity of biofoulers in predicting the success of shell fishes and fin fishes were demonstrated by Chagas et al. (2018) and Sievers et al. (2014).

The depth-wise fouling patterns were discussed by Cowie (2009). Svane (2006) worked on biofouling impacts and efficiency of anti-fouling trials in tuna sea cages in Australia. The basic information on fouler diversity, composition and succession are very much required to control the foulers and to estimate the fouling potency of that locality (Pati et al., 2015; Satheesh, 2006).

2.6 Biofoulers communities in relation to environmental parameters

Salinity, temperature, pH, dissolved oxygen and ammonia are considered as very important parameters in aquaculture practices (Pitta et al., 1999; Wu et al., 1999; Karakassis et al., 2001). The influence of physico-chemical parameters on *Tubularia larynx* (hydroid) was studied by Nellis and Bourget (1996). The blockages of meshes due to cage net fouling leads to the low dissolved oxygen level inside the cage. The community compositions of the biofoulers were reported to have significant correlation with the various environmental parameters (Fitridge et al., 2012).

Water quality studies of Indian cage culture sites were carried out by many researchers (Joseph et al., 2010; Philipose et al., 2010, 2012). Philipose et al. (2010) studied variations of environmental parameters of sea cage farm of Karwar in west coast. The correlation between the environmental parameters and biofoulers of the cage culture site is not available.

2.7. Influence for environmental parameter on biomass of biofoulers

Globally, sea cage farming population are busy in finding out cost effective methods to solve biofouling problem, environmental issues etc. and it found varied with the location and environment (Pudota, 2011; Braithwaith and McEvoy, 2005). Regarding the temporal distribution of biofouler biomass, along the east coast, highest biomass was

observed in November. Green mussels and barnacles are contributed the majority of the biofouling biomass (Sahu et al., 2011) on the wooden panels in jetty.

Chapter 3: Materials and Methods

Study area

The present study was carried out in marine cage farm of Karwar Research Centre of CMFRI during the cage culture period of the years 2014-2017. The study deals with the composition, distribution, density, diversity, biomass, succession and the influence of environmental parameters on biofouling organisms. In the present study, biofoulers on experimental net panels of two sites (2015-2016), culture nets and barrels were analysed during the study period.

3.1. Sampling site

The net panels were deployed in the cage farm of CMFRI, Karwar (N 14°48.406', E 074°06.664') in Arabian Sea, off Karwar along west coast of India (fig 3.1.1&3.1.2). Boat facility with safety equipments were used for sampling.

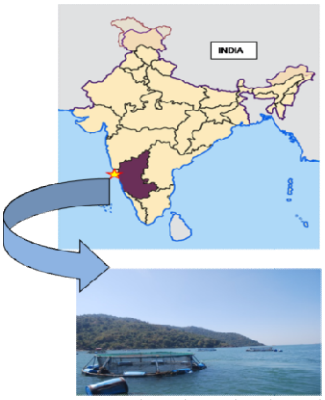


Fig. 3.1.1. Map showing the location of the Cage farm of CMFRI, Karwar in west coast of India (Source: Google earth maps)

Fig. 3.1.2. Map showing satellite view of the location of the cage farm of CMFRI, Karwar *(Source: Google earth maps, US Dept of state Geographer, Data SIO, NOAA, US, Navy. NGA, GEBCO@2016. Google IMAGE Landsat /Copernicus)*

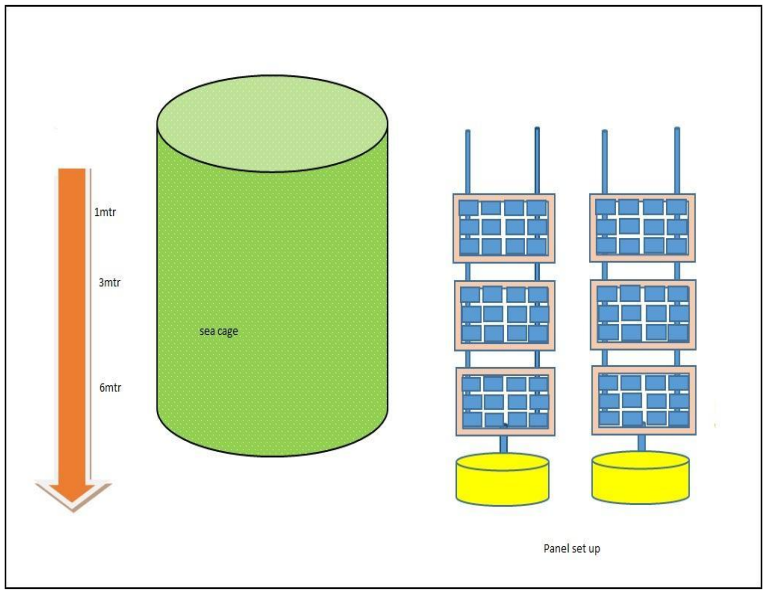
Site1 Cage site: This site the net panel set up is deployed inside the cage farm, where the experimental panels were placed in vertical position by tying them to the frame of the moored cage where culture is in progress.

Site 2 Reference site (control site): The reference site for the net panel experiment is selected 500 m. away from cage farm, where there are no cage culture activities are carried out (Cook et al., 2006). In this place, the panels were placed with the help of barrel, which was moored using a cement anchor and rope. It is termed as reference site rather than the control because the environmental parameters are not in control, but fish culture activities are not carried out in this site.

3.2 Sampling design:

The panels set up were made with HDPE net (net which is wildly used in open sea cage culture) were used for analysing the biofouling assemblage. The water quality parameters were recorded to find out the correlation between water quality parameters and biofouling.

Field panel set up and deployment: Panel fabrication was done with 12 square HDPE net panels fixed to half inch PVC pipe frame. This test frame is rectangular frame of length, 38cm X width, 50cm. The dimension of the PVC pipe is 19mm diameter, frame periphery is 176cm and intersections at 10cm. HDPE, nets ($10x10cm^2$) with a mesh size of 22mm were attached to the frame using a twine thread. A ladder of three sets (in 3depths: 1m., 3m., 6m.) were made by using 4mm diameter nylon rope (fig 3.2.1). Each series has 36 net panels i.e. 12 net panels in each depth. Total 72 net panels were deployed in two sites along with the duplicates. These panels were held firmly down in the water column using 5 kg weight. One panel each month from the 12 net panel setup was detached and brought to laboratory (Sahu et al., 2011) along the duplicates for analysis. 1m., 3m. and 6m. depth represented the top, middle and bottom portion of the cage net (fig 3.2.1).



*The orange colour arrow indicates the 3depths, Green cylindrical structure represents sea cage and orange rectangular structure represent the panel set up with blue net panels with weight (yellow color)

Fig. 3.2.1. Represents panel positions at three depths around the cage (orange rectangular structures represent the experimental panels and green cylindrical structure represents sea cage).

3.3. Field study:

3.3.1. Panel Sample collection: The detached net panels from two sites and 3 depths were brought to the laboratory separately in the plastic trough with sea water.

Fig.3.3.1. Experimental test panel sample collection of cage and reference site

3.3.2. Cage culture-nets sample collection:

Culture net samples are collected from cage nets during net exchange in the farm which was done on a monthly basis. During monthly net exchange, fouling data is collected for one culture period (9 months) during 2015-16 by random quadrate method. Randomly six quadrates of 10X10 cm^2 were selected and the foulers are collected and

brought to the laboratory for further analysis. Total density/biomass was estimated by random quadrate method.

Fig. 3.3.2. Net exchange

Fig. 3.3.3. Sampling during net exchange

Fig. 3.3.4. Fouling on net, sample

Fig.3.3.5. Cage barrel sample collection collected from culture net

During seasonal barrel exchange (four months once), the fouling data was collected during 2015-16 by random quadrate method. Randomly six quadrates of 10x10cm^2 were selected and the foulers are collected and brought to the laboratory for further analysis. Based on the study, total density/biomass was estimated.

Fig.3.3.6. Fouling on barrel

Fig. 3.3.7. Fouling samples collected from barrel.

3.3.3. Water sample collection:

Water quality parameters - temperature, salinity, dissolved oxygen, pH, ammonia, nitrite and phosphate of cage and reference site were analysed on monthly basis in the sampling stations, along with the biofouling samples (APHA, 1998).

Fig. 3.3.8. Water sample collection.

Laboratory studies

The net panels collected were washed separately with sterile sea water and fouling organisms were sieved in 200 micron sieve (Bloecher et al., 2013), then they were separated in to groups (biofouling communities), then they were used for further analysis and preservation.

Density (individuals/cm^2), biomass (g./cm^2) and percentage of major macro fouling organisms was studied by counting individuals, using Sartorius weighing balance, digital vernier caliper and photographic images. The organisms which were 2mm and below were counted using magnifying lens (colony counter and 4x magnification in Labline-Olympus microscope) with counting 1cm^2 area (10 random counts). Larger individuals were counted for 10x10cm^2 area and calculations were done accordingly. The colony forming individuals were counted by considering each colony as single unit (Cook et al., 2006; Pati et al., 2015). The identified foulers were classified to different community /group (Sahu et al., 2011; Cook et al., 2006; WHOI, 1952).

Fig.3.3.9. Fouling sample analysis in laboratory

The samples were preserved in Rose-bengal and 5% formaldehyde for further identification. Smaller organisms were observed under 5x magnifications in AXIO, Zeiss (Scope-A1) microscope. Identifications up to genus and species level are done by using the different keys (details mentioned in 3.4).

3.3.4. Data Analysis

Statistical analysis: The data collected was analyzed statistically using SPSS 16.0, PAST 2.14, PRIMER 0.5 and XL-STAT softwares to derive abundance, indices, similarity, correlation and compared using two way ANOVA for assessment of diversity of organisms (Pati et al., 2015)

3.4. Chapter wise methodology

3.4.1. Composition and distribution of biofoulers

To find out the composition of the biofoulers, the count of number of organisms was taken. Seasonal data of all the biofouling communities are pooled to find out community composition of biofoulers in the study locations.

Biofoulers were broadly classified into Phylum. Identifications up to genus and species level are done by using the different keys (WHOI, 1952; Worms, 2016; Chambers and Muir, 1997; Conlan, 1989; Hayward and Ryland, 1995).

The fouling organisms were grouped as communities (WHOI, 1952) for further studies. Here after the analyses are based on the “biofouling communities”.

3.4.2. Monthly and seasonal variations in density of biofoulers:

The density of the biofoulers is based on the number of individuals counted on the 100cm^2 net panel excluding the PVC frame. The depth wise collection of the biofoulers on the panels were done and monthly samples collected were sorted washed, photographed and preserved in 10% formalin.

The seasonal density was based on the 3 seasons, pre monsoon (Feb-May); monsoon (June-Sept); post monsoon (Oct-Jan). ANOVA test was carried out to find out the significance between the biofouling communities, season and site using SPSS software (version16.0).

3.4.3. Monthly and seasonal variations in biomass of biofoulers

To find out the biomass of the biofoulers, the total wet weight of each biofouling communities was taken using the Sertorius weighing balance. The biomass (g./100cm^2) of all the fouling communities present on the net panel were analyzed (Sahu et al., 2015). The seasonal composition of the cage net panel, reference net panels, cage net and the cage barrel were evaluated using the seasonal data (Cook et al., 2006).

3.4.4. Community succession of biofoulers

For community successions studies two sites were chosen so as to compare between cage and reference. The experimental net panels were installed in marine farm and the reference site during the period December-2015 to November-2016. Every month three panels (1m., 3m., 6m. depth) from each site were brought to laboratory for analysis. Monthly, seasonal data of culture net and barrels were collected for further analysis.

Average data of the panels from three depth of each site was taken for finding the succession pattern, climax species and comparison of settlement pattern between the sites (Greene and Grizzle, 2007; Sahu et al., 2011; Pati et al., 2015). The data collected was analyzed statistically using software like PAST-2.14, PRIMER-0.5 and XL-STAT-2016.

3.4.5. Diversity indices of biofoulers

The diversity of biofouling community was assessed using diversity indices: Dominance (D), Simpson Diversity index (1-D), Shannon (H), Evenness index (e^H/S), Margalef index (species richness). The diversity indices of the net panels, culture net and barrel were analyzed during the each net and barrel exchange.

Statistical analysis: The statistical software's Primer, PAST, SPSS, XLSTAT, MS excel were used for computing results. Graphs for diversity index were computed in PAST version 2.14 (Pati et al., 2015) and were subjected to statistical analysis done by one-way ANOVA. Seasonal indices of all the locations are also calculated using the Primer 6.1 software version.

3.4.6. Biofoulers communities in relation to environment

Physico-chemical parameters like temperature, salinity, dissolved oxygen, pH, ammonia, nitrite, phosphate were estimated by standard methods (APHA, 1998). Temperature was measured by mercury thermometer (±0.01^{0}C accuracy) and salinity was measured by Salinometer (Atago, Japan). Dissolved oxygen estimation samples were

done by Winkler's method and pH, was measured by using the pH probe (Multi parameter-Test-35-series). The nutrients ammonia, nitrite, and phosphate were estimated by using colorimetric (Strickland and Parsons, 1972) and Spectrophotometeric method. Monthly and seasonal variation in salinity, temperature, dissolved oxygen, pH, ammonia, nitrite and phosphate were studied in all the three depth where the experimental net panels were deployed at cage and reference site (Sahu et al., 2011; Pati et al., 2015).

The correlation with the seasonal variation of hydrography, fouling number, fouling diversity and fluctuations in the growth of fouling organisms were studied. The detailed study about the correlation between each community and the water quality parameter is further studied (Sahu et al., 2015) using the two tailed Pearson correlation analysis, using SPSS software. Correlation between environmental parameters and diversity indices of fouling communities were analyzed (Pati et al*.,* 2015) in PAST software. The results obtained using CCA of relationship between the water quality parameters (salinity, temperature, dissolved oxygen, pH, ammonia, nitrite, phosphate) and biofouling community indices (dominance, diversity, evenness and total abundance) are presented in graphs and tables.

3.4.7. Influence of environmental parameter on biomass of biofoulers

The biomass of the biofoulers was based on the weight of the biofouling organisms community wise from the net panel, weighed in the Sertorius weighing balance. Water quality parameter salinity, temperature, dissolved oxygen, pH, ammonia, nitrite and phosphate were analyzed using standard methods (APHA, 1998). Biomass (g./100cm^2) and water quality parameters on the experimental net panels were correlated using correlation coefficient (Pearson) using PAST (ver 2.14).

Chapter 4: Results and Discussion

4.1. 1.Biofoulers composition and distribution

Total of 23 biofouling communities with 57 species belonging to 11 phyla were found in the present study. Arthropoda was the most diverse phylum with highest number of species (14) and phylum rhodophyta was represented by the lowest number of species

(2) (fig 4.1.1). Totally 20 classes, 32 orders and 48 families have been observed from the study site. Among these, decapoda was the largest order recorded, possessing 7 species and contributing 13% of biofouling diversity. Families ostreidae and nereididae, had the maximum of 3 species and ulvaceae, bugulidae, surpulidae and mytilidae had 2 species each, rest all families had 1 species each (fig 4.1.1).

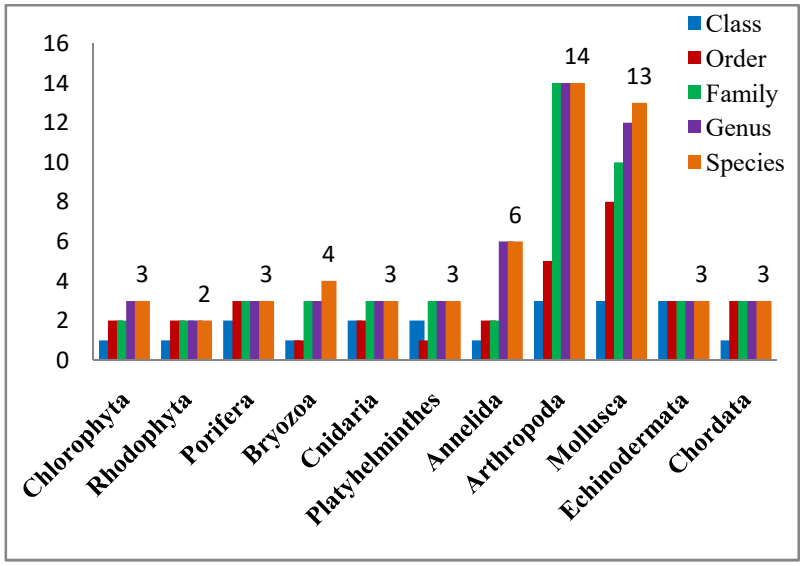


Fig. 4.1.1. Species distribution in phylum of biofoulers

Table 4. 1. 1.a. Check list of biofoulers identified in the present study

Sl No	Biofouling communities	Species
1	Algae	*Enteromorpha intestinalis (*Linnaeus) Nees,1820) *Enteromorpha compressa* (Linnaeus) Nees,1820) *Chaetomorpha antennina* (Bory) Kutzing,1847) *Grateloupia lithophila* (Borgesen,1938) *Ceramium cruciatum* (Collins & Hervey,1917)
2	Sponges	*Scopalina sp.* (Schmidt, 1862) *Plakortis sp.* (Schulze, 1880) *Halichondria panacea* (Pallas,1766)
3	Bryozoans	*Bugula neritina* (Linnaeus,1758) *Bugula simplex (*Hincks,1886) *Cryptosula pallasiana* (Moll,1803) *Triphyllozoon moniliferum* (MacGillivray,1860)
4	Sea anemones	*Bunodosoma cavernatum* (Bosc,1802)
5	Hydroids	*Obelia bidantata* (Clark,1875) *Salacia tetracythara* (Lamouroux,1816)
6	Flat worms	*Leptoplana sp.* (Montagu, 1815) *Stylochus sp.* (Eehrenberg, 1851)

		Prostheceraeus vittatus (Montagu,1815)
7	Polycheate worms	*Platynereis dumerilii* (Audouin &Milne- Edwards,1834) *Perineris cultrifera* (Grube,1840) *Hydroides elegans* (Haswell,1883) *Pomatoceros triqueter* (Linnaeus,1758) *Sebella sp.* (Linnaeus,1767) *Ceratonereis costae* (Grube,1840)
8	Amphipods	*Hyperia sp.* (Latreille, 1823) *Caprella sp. (Lamarck, 1801)* *Gammarus locusta* (Linnaeus (1758)
9	Barnacles	*Balanus amphitrite* (Darwin,1854)
10	Crabs	*Schizophrys aspera* (H.Milne Edwards,1831) *Ozius rugulosus* (Stimpson,1858) *Dromia dromia* (Linnaeus, 1763) *Grapsus strigosus* (*Herbst, (1799)*) *Pisidia longicornis* (Linnaeus,1767) *Medaeus granulosus* (Haswell,1882)
11	Isopods	*Paranthura* sp. (Bate and Westwood,1866) *C.fluviatilis*(Stebbing,1902)
12	Shrimps	*Alpheus malabaricus* (J.C.Fabricius,1775)
13	Pycnogonids	*Endeis mollis* (Carpenter,1904)
14	Gastropods	*Euchelus atratus(*Gmelin,1791) *Archidoris pseudoargus* (Rapp,1827)
15	Green mussels	*Perna viridis* (Linnaeus,1758)
16	Scallops	*Pinctada fucata* (Roding,1798) *Acanthochitona crinite* (Pennant,1777) *Anomia simplex* (d'Orbigny,1853)
17	Modiolus	*Modiolus metcalfei* (Hanley,1843)
18	Nudibranchs	*Hermissendra crassicornis* (Eschscholtz,1831)
19	Oysters	*Crassostrea madrasensis* (Preston,1916) *Crassostrea gryphoides* (Schlotheim,1820) *Saccostrea cucullata* (Born,1778)
20	Limpets	*Patella radiata* (Perry,1811) *Diodora aspera* (Rathke,1833)
21	Ophiuroids	*Ophioderma sp.* (Miille & Troschel, 1840) *Antedon sp.* (De Feminville, 1811)
22	Echinoids	*Stomopneustes variolaris* (Lamarck,1816)
23	Ascidians	*Ciona intestinalis* (Linnaeus,1767) *Molgula occidentalis* (Traustedt,1883) *Didemnum psammathodes* (Sluiter,1895)

4.1.2. Distribution of biofoulers

The percentages of biofoulers collected from all the sites were compared. The percentage distribution of different phyla is given in fig 4.1.2.

In overall, phylum cnidaria formed the major portion of biofouling composition. At the cage site and the reference site, 9 phyla have been recorded from the experimental test panels. Algal groups; chlorophyta and rhodophyta were absent on these panels. Species belonging to phylum Tunicate was present in all the sites, except on cage barrels. Highest composition of cnidarian biofoulers were on the cage barrels (83%).

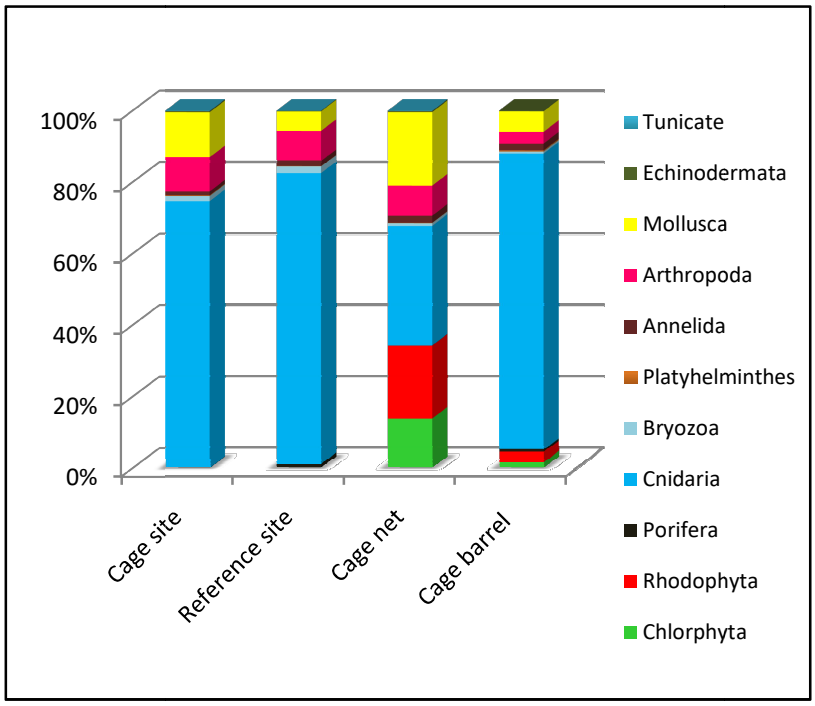


Fig. 4.1.2. Distribution of biofoulers on different substrates.

4.1.3. Biofouling communities distribution:

Highest numbers of biofouling communities were recorded on “cage culture nets” (22 communities). Except scallops almost all the other communities were found on the nets, which were exchanged regularly. Sponges, hydroids, bryozoans, polychaete worms, amphipods, barnacles, crabs, isopods, green mussels and *Modiolus* were the common biofouling communities found in all the sites of the study area.

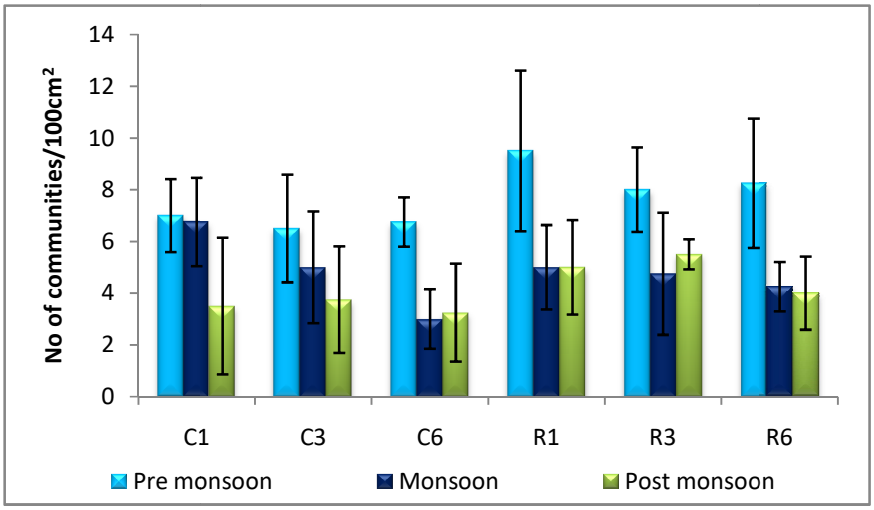


Fig.4.1.3. Seasonal biofouling communities (Mean ± SD) in two sites and 3 depths

The highest number of communities were found in the reference site during pre monsoon season (10±3 communities/100cm^2) and lowest were found in the cage site during monsoon 6m. depth panel (3±1 communities/100cm^2) (fig 4.1.3). One way ANOVA test was conducted in PAST software the results showed significant difference between the season (One way ANOVA, F=15.36, P<0.0005). The turkeys pair way comparison test revealed that, there is a significant difference between the communities of pre monsoon and post monsoon season (p<0.0005) and pre monsoon and monsoon season (p<0.005).

Discussion

Overall, 11 phyla were recorded in the present study, since this is a pioneering attempt, for making a comparative statement, there were no reports available on species composition of biofoulers from cage culture farms from India. Similar study conducted in salmon farm in Norway, species belonging to 12 phyla were recorded on the nylon net panels (Pudota, 2011), even though phyla recorded from Norway was more, the number of species recorded was lower than those in the present study. Five common fouling taxa found on the cage net in the present study were algae, cnidaria, bryozoa, mollusca and crustaceans, which were reported in most of the works elsewhere also (deNys and Guenther, 2009; Braithwaite and McEvoy, 2005; Kassah, 2012). In the present study hydroids were most common macrofoulers through out the study period. Occurance of

hydroids and its significant variations with depth in culture cage-nets were reported by Kassah (2012), Nellis and Bourget (1996) and Guenther et al.(2010).

Guenther et al. (2010) and Pudota (2011) described hydroids (*Ectopleura larynx*), blue mussel (*Mytilus edulis*), algae (green and red), amphipods, nudibranchs (*Aeolidia papillosa* and *Coryphella sp*.) were the important biofouling groups on the net panels studied in aquaculture sites in Norwegian water. Compared with those studies, present study recorded more number of species. In the present study, hydroids (*Obelia bidantata*), green mussel (*Perna viridis),* barnacles *(Balanus amphitrite)*, horse mussel *(Modiolus metcalfei)* were the important species in both the sites and cage substratum including net panels of all the depths.

Minimum number of biofouling communities in the cage site during monsoon may be because of the degradation of most of the marine biofouling communities due to the fresh water influence by Kali river influx in monsoon season.

From the seasonal analysis, it was found that, in all the seasons hydroids dominated the panels, but the percentage composition of the hydroids as well as other biofouling communities varied in all the seasons and it is specific for the site and the depth. During the post monsoon, the hydroid percentage composition was less compared to the pre monsoon and monsoon. During monsoon, barnacle percentage was noticeably high because those biofoulers could withstand the salinity variations. *Modiolus* and green mussels showed rise in the percentage composition representing the spat fall in the post monsoon season. Flat worms and ascidians were found only in the monsoon season in the cage site. Sahu et al. (2015) studied the biofoulers at Kalpakkam waters near the Jetty, using different panels and they also recorded similar fluctuations of the biofoulers as in the present study.

4.2 Monthly and seasonal variations in density of biofoules:

4.2.1. Monthly total density variations of biofoulers:

The population density of biofoulers in cage site ranged between 20±18.32 individual/100cm^2 (June-16) to 3050±149.91 individuals/100cm^2 (August-16) and at the reference site density varied between 21±2.83 individuals/100cm^2 (July-16) to 4167±59.40 individuals/100cm^2 (May-16). The number of individuals of all the

communities represents the total density of biofoulers in two sites in 3 different depth panels during different months.

The statistical "t test" for density of all the depths were done in PAST for monthly data of mean density and the results are presented. The monthly variations of mean density of biofoulers are significantly higher in cage 6m. depth panel and reference 1m. depth panel ($p<0.0002$).

4.2.2. Average density of biofoulers in cage and reference site:

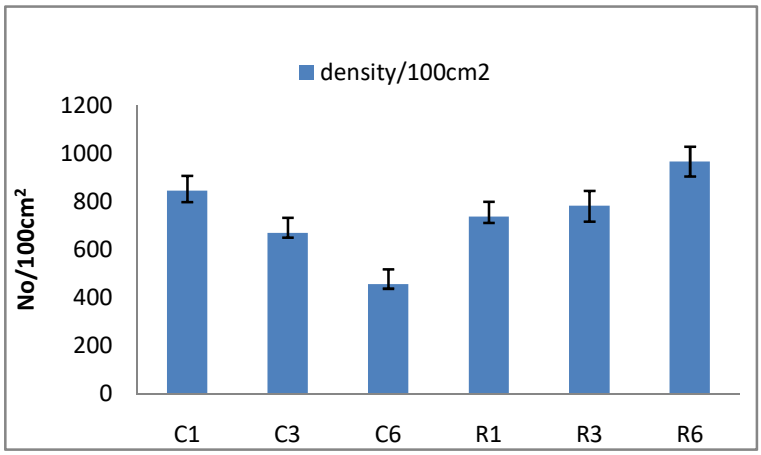


Fig. 4.2.1. Depth wise average density of biofoulers on the experimental net panels of cage and reference site. (Error bars indicate the standard deviation).
[C1-cage, 1m. depth panel; C3-cage 3m. depth panel; C6-cage 6m.depth panel; R1-reference, 1m. depth panel; R3- reference, 3m.depth panel and R6-reference, 6m.depth panel]

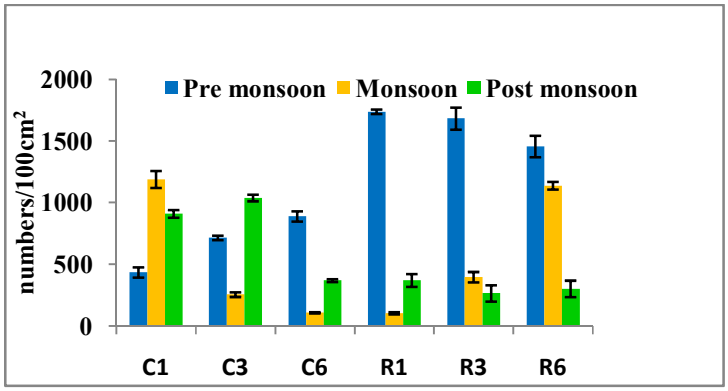


Fig .4.2.2. Depth wise seasonal density on the experimental net panels of cage and reference site. (Error bars indicate the standard deviation).

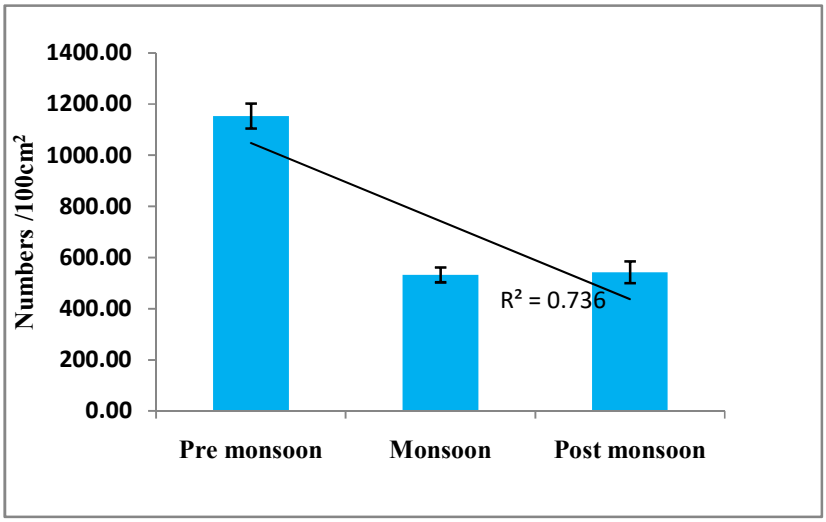


Fig. 4.2.3. Seasonal density of biofoulers. Error bars indicate the standard deviation.

The ANOVA results obtained from SPSS, for site and biofouling communities has showed that there is no relationship between the sites and density of biofouling communities ($p>0.05$), but between the months there is a significant relationship ($p=0.005$). The seasonal ANOVA results revealed bryozoans and isopod communities have shown positive relation with high significance between the seasons ($P<0.05$ and $P<0.01$). The pot hoc results revealed that bryozoans showed positive relation between the pre monsoon and post monsoon season ($p=0.015$) and isopods showed higher significance between the pre monsoon and monsoon season ($p=0.009$).

Discussion

High density of biofoulers were recorded in the reference site (828.40±15.01 number/100cm^2), compared to the cage site panels (656.97± 29.11number/100cm^2), which was away from the cage site. The variation among the different sites is usually depended on the light penetration, water flow, depth and location of the structure (Guenther et al., 2010; Fitridge et al., 2012). The general studies in Indian west coast if compared, the panels made of 'Perspex' at west coast of India in Jetty have showed a higher average density (17298 number/m^2)in pre monsoon and lower density during monsoon (Swami and Udhayakumar, 2010). Present results also revealed that over all biofouling density is higher in pre monsoon and lower in monsoon (fig 4.2.3) and monthly density was higher in February and lowest in June (Swami and Udhayakumar, 2010). In present study, highest and lowest population density of biofoulers on

experimental net panels ranged between 20 ±18.32 to 4167 ± 59.40 individuals/100cm^2 during June-16 to May-16. The cumulative studies carried out at south east coast of India by Sahu et al. (2011) ranged between 63 to116x10^3 numbers/230cm^2 i.e. 0.27 to 722 numbers/cm^2 on wooden panels and 12 to 69 numbers/cm^2 on monthly panels. Since material used is different and surface area is less on the net panels, the present varying range in density might have been observed in these cumulative studies.

The ANOVA results for the density of biofouling communities has varied with the season showing significant difference ($P<0.05$). Some of the biofoulers, bryozoans and isopods showed positive correlation of statistical significance between the seasons, influencing the structure of the biofouling community. In short term biofouling studies in southeast coast of India, bryozoans have not shown defined trend of temporal variation in settlement on teak panels (Sahu et al., 2011), but in present study the bryozoans and isopods showed ($p<0.05$) significance between the season also there is correlation between the bryozoans and isopods. In the general fouling studies carried out in Kalpakkam water (Rajagopal et al., 1990) bryozoans were noticed in post monsoon months. In the present study also bryozoans appeared in post monsoon season. A similar study carried out at Europe in cage farm, dominance of bryozoans biofoulers observed in reference (Cook et al., 2006) and they suggested that these biofoulers dominated due to their higher competiveness.

4.3. Monthly and seasonal variations in biomass of biofoulers

Biofouling was observed in all the experimental net panels of all the months and depths of cage and reference site. The highest biomass of 685.74 ±89.02g./ 100cm^2 was observed in November-16 at 6 m. and lowest was, 0.03±0.03g./100cm^2 recorded in June-16 at 3m. (fig 4.3.1). In the reference site, higher biomass values were found in the 3m. depth panel and highest was during September-16 (74.33±14g./100cm^2), which was due to green mussels (fig 4.3.1.).

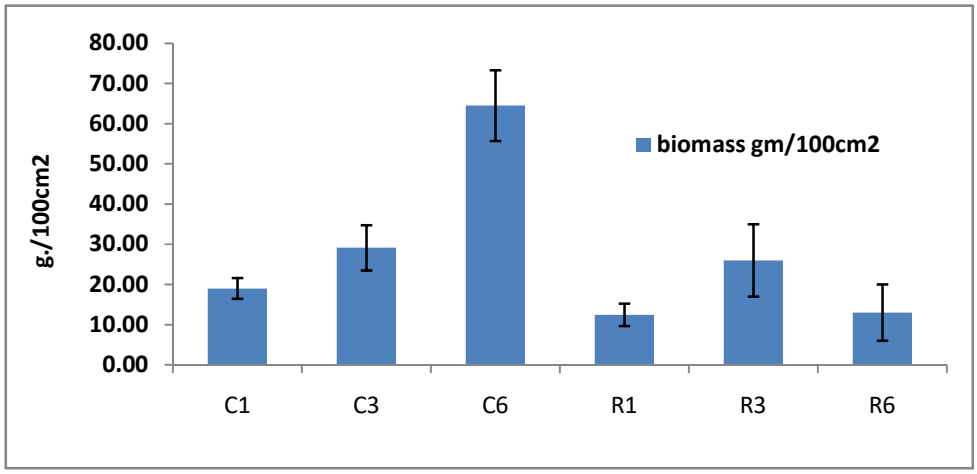


Fig. 4.3.1. Average biomass of biofoulers (g./100cm^2).Error bar indicates the ±SD.

[C1-cage,1m depth panel; C3- cage, 3m. depth panel; C6-cage, 6m.depth panel; R1-reference,1m. depth panel; R3- reference, 3m.depth panel and R6- reference, 6m.depth panel]

4.3.1. Seasonal biofouling biomass

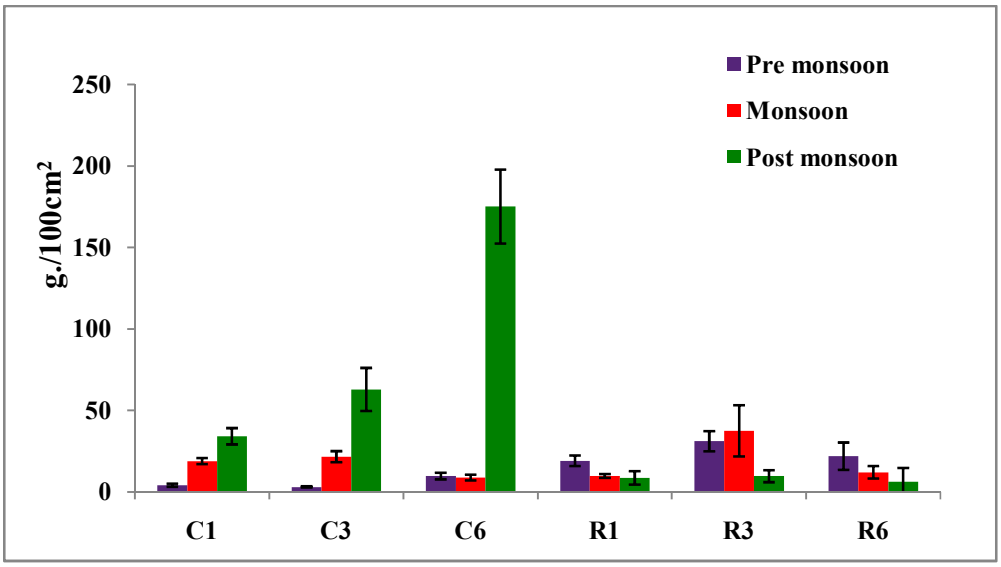


Fig. 4.3.2. Seasonal biofouling biomass (g./100cm^2) on experimental test panels. Error bar indicates the ±SD.

In overall, as far as experimental test panel studies were concerned, the maximum biomass (175.04±22.67g./100cm^2) was observed in 6m. depth, cage panel during post monsoon season and minimum (3.02±0.36g./100cm^2) in 3m. depth cage panel during pre monsoon.

Discussion

In the present study, maximum biomass was observed at the cage site panels with 685.74g./100cm^2 during November-16 (12 month panel) and highest biomass at the reference site was during September-16 (10 month panel) with 74.33g./100cm^2. The similar result was obtained by Cook et al. (2006), they recorded higher biomass on the artificial structures at the mariculture site than in reference. Completely different community biomass and composition were recorded at cage site and reference, which was also true in present study. The suggestions of earlier authors that the higher food availability at the mariculture site, and the high plankton productivity due to higher nitrogen obtained at cage site will enhance the higher community biomass on the cage culture structures in cage site is supportable. In general the less biomass on the reference experimental net panel may be due to grazing (Pati et al., 2015) and due to less food availability (Cook et al., 2006).

During the twelve months of deployment, the highest infestations were nearly nine times greater biomass which was observed in the cage site than the reference site. In view of Cook et al. (2006), the biomass increase was 7 times at mariculture site than the reference site. In present study, India being the tropical country the higher biofouling biomass might have been obtained. There is no significant difference between the two sites.

During monsoon and the minimum biomass were on the culture net. The similar results were reported by Cook et al. (2006), they recorded higher biomass on the artificial structures at the mariculture site than from reference. In some season, the biomass and composition of biofoulers recorded at cage site and the reference were widely diverse, similar cases were also observed in the present study.

4.4. Community succession of Biofoulers

4.4.1. Monthly settlement of biofoulers :

4.4.1. 1.Experimental net panels on the cage site:

The results of dominant biofoulers on the experimental net panels attached to cage and in the reference site are represented in the fig 4.4.1 & 4.4.2**.**

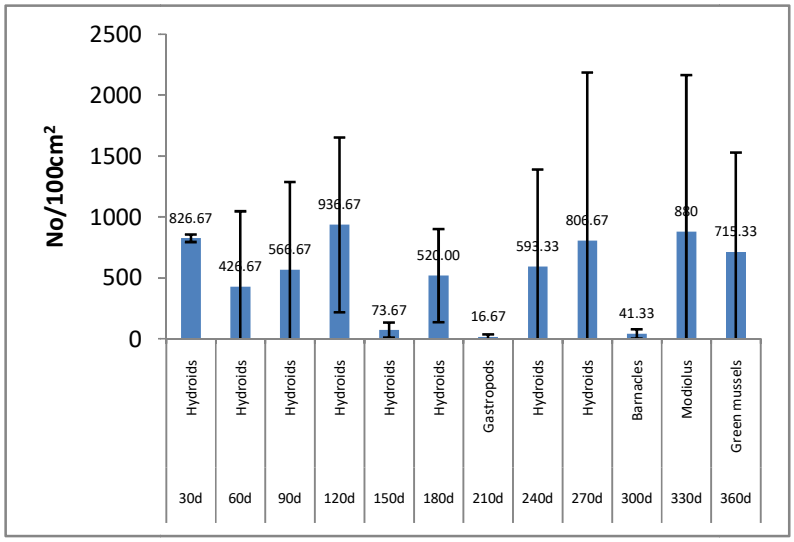


Fig. 4.4.1. Graph showing dominant biofoulers on the experimental net panels of cage site

4.4.2.2. Experimental test panel in the reference site.

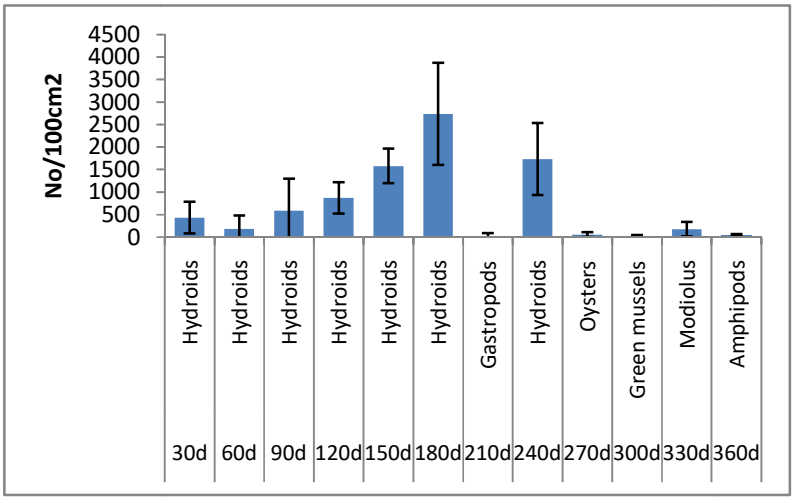
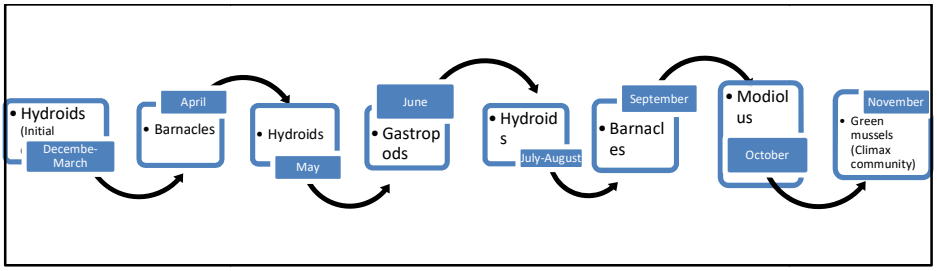
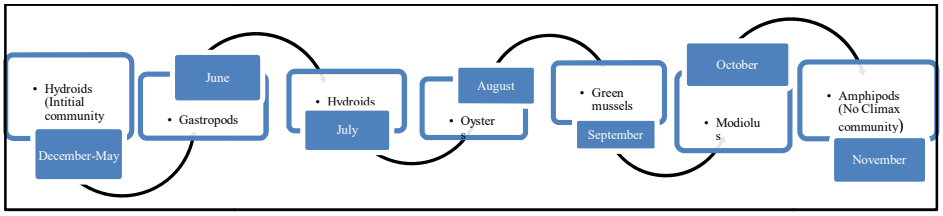


Fig.4.4.2. Graph showing dominant biofoulers on the experimental net panels of reference site.

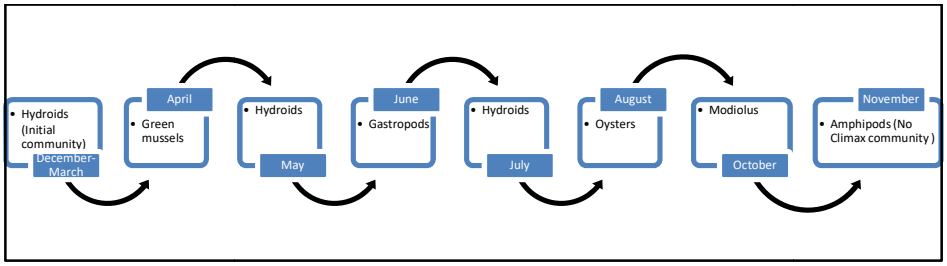
The fig 4.4.2. represents the dominant biofouling communities in reference site during this period.



a. succession pattern on the long term panels of cage site



b. Succession pattern on the long term panels of reference site

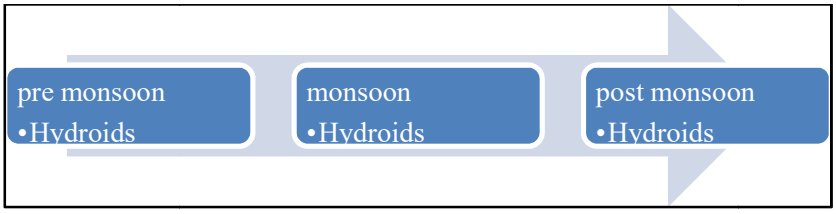


c. Succession pattern on the short term cage culture net

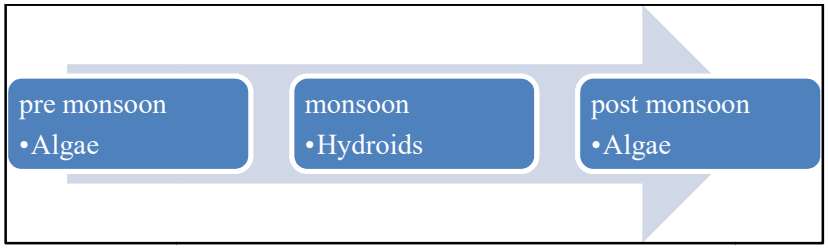
Fig. 4.4.3 . Monthly (a-c) Succession pattern of biofoulers

4.4.3. Climax community: Green mussels (*Perna viridis*) were the dominant fouling community forming the climax in the cage site net panels after 12 month of immersion. But in the reference site, the climax community was not so prominent (fig 4.4.3).

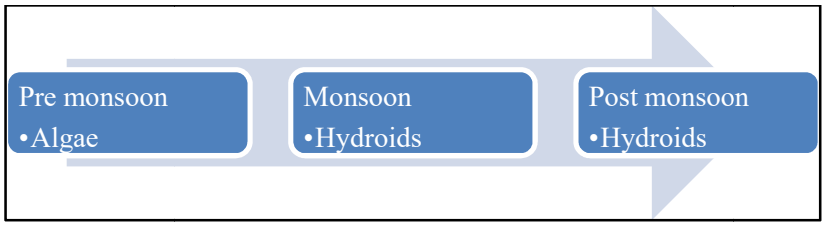
Fig. 4.4.4. Green mussels forming the climax on the panel in sea cage farm



d. Seasonal Succession pattern on the long term panels (cage site and reference)



e. Seasonal Succession pattern on the short term cage culture net



f. Seasonal succession pattern on the barrel

Fig. 4.4.5. Seasonal succession pattern (d-f) of biofoulers

Over all algae and hydroids were the primary settling communities, gastropods are grazing communities, barnacles, oysters, *Modiolus* are the intermediate settling communities and green mussels formed the climax communities (fig 4.4.6).

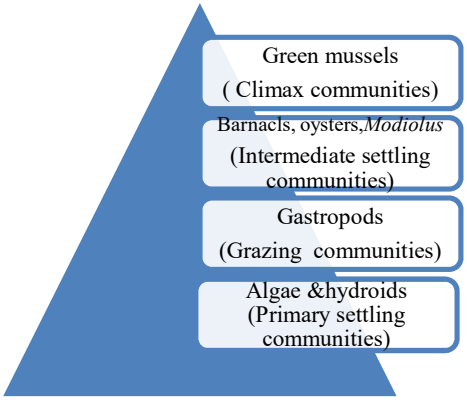


Fig. 4.4.6. Biofouling pattern of cage farm

Discussion

The results of the present study can be compared with the biofouling studies in bivalve aquaculture (oyster culture), wherein initial colonies included were hydroids, bryozoans, sponges, ascidians, polychaete worms, bivalves, barnacles and algae (Chagas et al., 2018; Durr and Whal, 2004; Sievers et al., 2014). In the present study hydroids and barnacles appeared in the initial month in the cage panel, whereas hydroids, polychaete worms, amphipods, barnacles, crabs, shrimps, isopods, green mussels, *Modiolus* and oysters appeared in the reference panel. The recruitment, settlement complexities could be the

reason for these differences in marine invertebrates (Keough, 1983; Chagas et al., 2018). The similar reasons could be given in the case of fin-fish aquaculture cage panel and the reference panel. The biofouling succession as well as colonization patterns differs with climatic zone, as in tropical zones constant settlements around all the months where as at fixed intervals in temperate zone (Fitridge et al., 2012, Chagas et al., 2018). The local surveys are necessary for the site selection of cage farming as the colony patterns of biofoulers differ along the farming areas (Chagas et al., 2018).

Succession on reference panels showed hydroids were the first settlers followed by gastropods and oysters in August-16, green mussels in June-16, September-16, *Modiolus* in October-16 and amphipods in November-16 (the climax community was not so prominent, may be due to the fact that it was in open waters. The grazing and predation were reported to be the factor which is affecting the dominancy and the climax communities (Sahu et al., 2011) of biofoulers.

In the seasonal succession pattern of biofoulers settlement of short term studies are different from long term studies, similar observations were made by Sahu et al. (2011). During monsoon low fouling diversity and density were found (Sahu et al., 2011) due to low salinity and low temperature. On the experimental net panels, peak settlement was during May-16 in reference site with hydroids forming highest total abundance but in cage site it is in November-16 (green mussels). Overall, in the May-16 fouling abundance was more on the cage nets.

In literature of **'**Ecological succession', Clements (1936) theory is an idealistic theory proposing climax as the final stage of succession process. In the temperate regions of the world, mussels (*M. edulis*) and ascidians (*Ascidiella aspera*, *Ciona intestinalis*) are reported to be the dominating climax communities (Hodson et al., 2000; Claere et al.,1994). Most of the Indian studies, climax species is *Perna viridis*, few studies barnacles and ascidians were climax communities Sahu et al. (2011). Very little literature only available on the "climax communities" concerned to the aquaculture net panels. Sahu et al. (2011) has reported green mussels as climax community on wooden panels. In the present study, which was carried out in culture farm, the green mussels formed the

climax community (fig 4.4.4). But in the reference site, which is away from the culture activities no climax as such was observed. This may be due to the grazing and predation effect of wild fishes and other animals in the reference site.

The pattern of colonization was studied in the Mediterranean in an IMTA system and the similar results were obtained and the mussel species *Mytilus galloprovincialis* was found to be the dominant fouler (Giangrande et al., 2020) in the study. Green mussels were the dominant foulers forming the climax, due to their higher efficiency to hold the net fibers, fast growth and higher ability to filter the phytoplanktons which are available in large quantity due to the higher nutrients availability. The other foulers get less place to survive, so not able to establish on the net (Sahu et al., 2011). The dominant organisms are successful due to their size, growth, longer life span, longer larval stage (Richmond and Seed, 1991). In most of the aquaculture related biofouling studies carried out in Mediterranean where sea bass is culture fish and mussels, hydroids, algae were the dominating communities (Fernandez-Gonzalez and Sanchez-Jerez, 2017) as shown in the present study.

4.5. Diversity indices of biofoulers

4.5.1. Monthly variations of diversity indices of biofoulers between cage and reference site.

In the results of monthly biodiversity indices - Dominance (D), Simpson diversity index (1-D), Shannon (H), evenness index (e^H/S), margalef index (species richness) were presented. The ANOVA results showed no significant difference between the indices of two sites ($p>0.05$).

Results of monthly biodiversity indices at cage site were presented in fig 4.5.1-4.5.4.

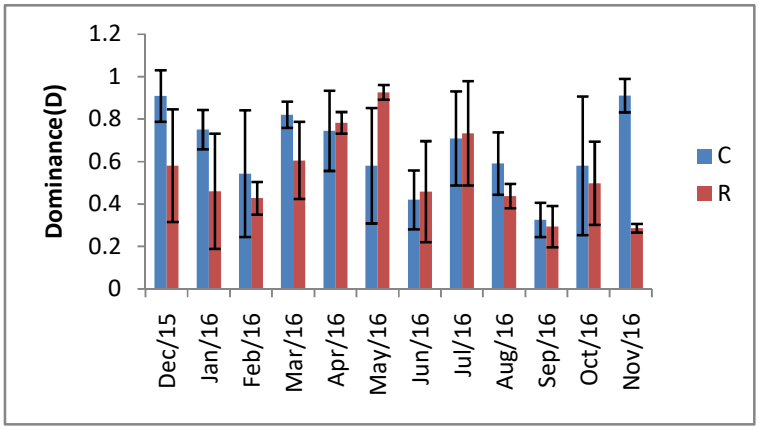


Fig. 4.5.1. Mean (± SD), Dominance(D) of the biofoulers at cage and reference site.

* C-cage site, R- reference site.

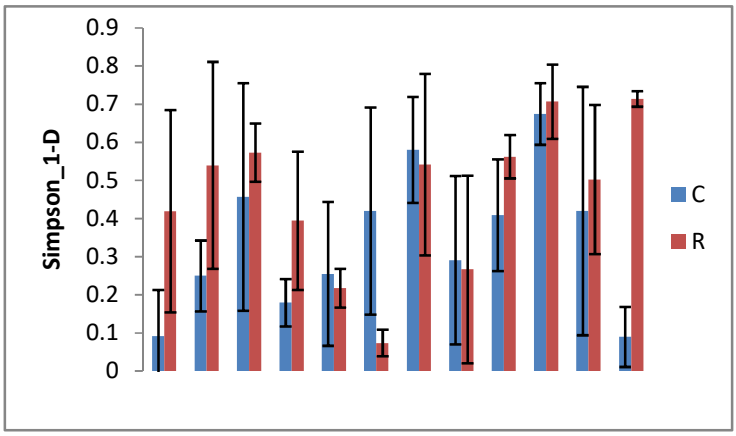


Fig. 4.5.2. Mean (± SD), Simpson dominace index(1-λ) of the biofoulers of cage and reference site.

* C-cage site, R- reference site.

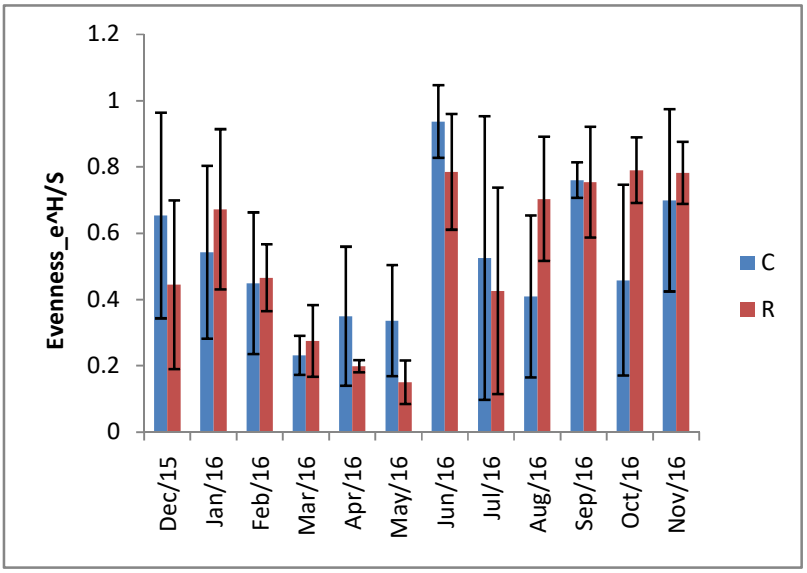


Fig. 4.5.3. Mean (±SD), Evenness index(e^H/S)of the biofoulers of cage and reference site

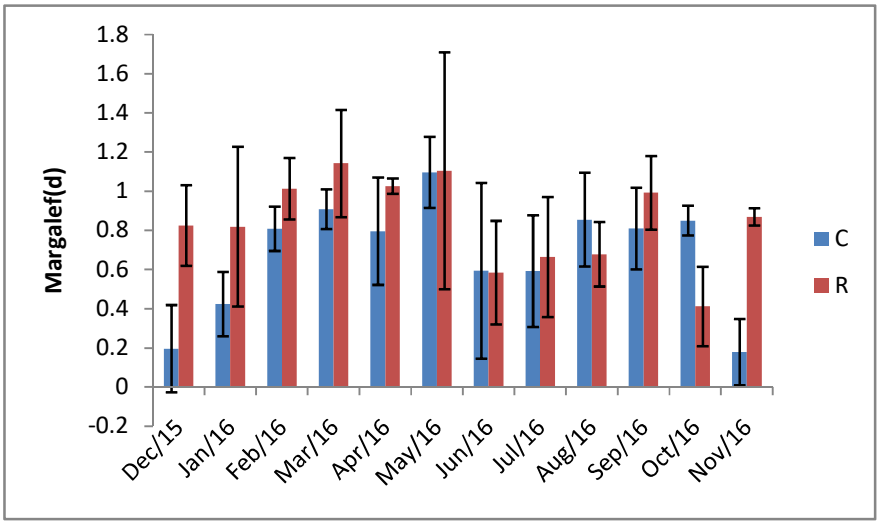


Fig. 4.5.4. Mean (±SD), Margalef richness index(d) of the biofoulers of cage and referance site (Error bars indicate the standard deviation). * C-cage site, R- reference site.

4.5.2. Overall biodiversity indices of cage and reference experimental net panels between three depths

Results have shown that highest Shannon-wiener index (H'log 2) of the biofoulers was observed in cage site, at 3m. depth (2.13±0.01) followed by 6m. depth (2.00±0.01) and reference site 1m. depth (1.98±0.11). Margalef's richness index was the highest in 6m. depth (1.28 ±0.04) of cage site followed by 3m. depth (1.22±0.03) cage site and 1m. depth (1.19±0.01) reference site. Simpson dominance index was the highest in cage 3m. depth (0.86±0.001) followed by reference, 1m. depth (0.83±0.003) and the lowest at reference 3m.(0.75±0.003). Evenness was the highest in cage, 3m. panel (0.70±0.001) followed by cage 6m. panel (0.61±001) and reference,1m. panel (0.60±0.007).

Discussion

Several workers reported that there is significant variation of assemblege of foulers on the cage net with depth (Kassah, 2012; Svane et al., 2006; Nellis and Bourget, 1996). In the present study the diversity indices varied on culture net according to depth and months. Results of diversity indices of biofoulers obtained from the present study were discussed and compared with similar study results of Elsewhere (Magurran, 1988; Pati et al., 2015; Bloecher et al., 2013; Braithwaite and McEvoy, 2005; Kassah, 2012).

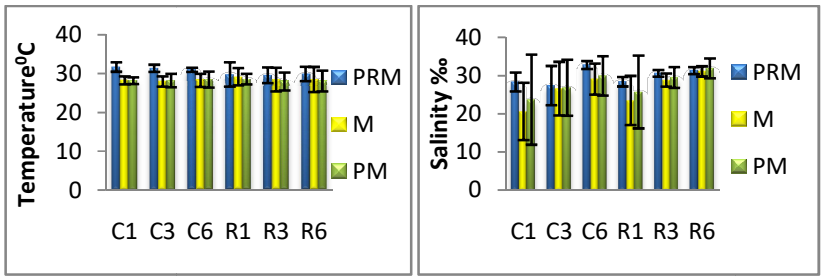
As reported by Magurran (1988) and Pati et al. (2015), dominance of biofouling community showing a reverse trend with evenness and diversity, was observed in the present study also. At cage site, 1m. depth, the dominance index was high and diversity is less during December-16, while species dominance was less and diversity is more in the month of June-16. It is well known that the dominance index decreases as diversity increases (Pati et al., 2015). The result was found true when Simpson and Shannon diversity indices were computed. Pati et al. (2015) suggested that diversity indices of biofouling community i.e. total abundance, species richness, diversity, evenness and density were relatively high in higher salinity, where as the indices were low when ammonia, nitrite, phosphate were present.

Seasonal studies of biodiversity indices of biofoulers were studied in the present research. The seasonal intensity of biofouling in the east coast waters of Kalpakkam was high in pre monsoon and low during monsoon (Sahu et al., 2015). The similar results were obtained in present biofouling study. Maximum dominance was in 6m. depth cage panel during pre monsoon and minimum dominance was in barrel during the same period. The basic information on fouler diversity, composition and succession is very much required to control the foulers and estimate the fouling potency of that locality (Pati et al., 2015 and Satheesh, 2006).

4.6. Biofoulers communities in relation to environment

4.6.1. Seasonal variations of hydrological parameters at cage and reference experimental test panel

Seasonal variations of hydrological parameters at cage and reference experimental test panel are presented in fig 4.6.1.



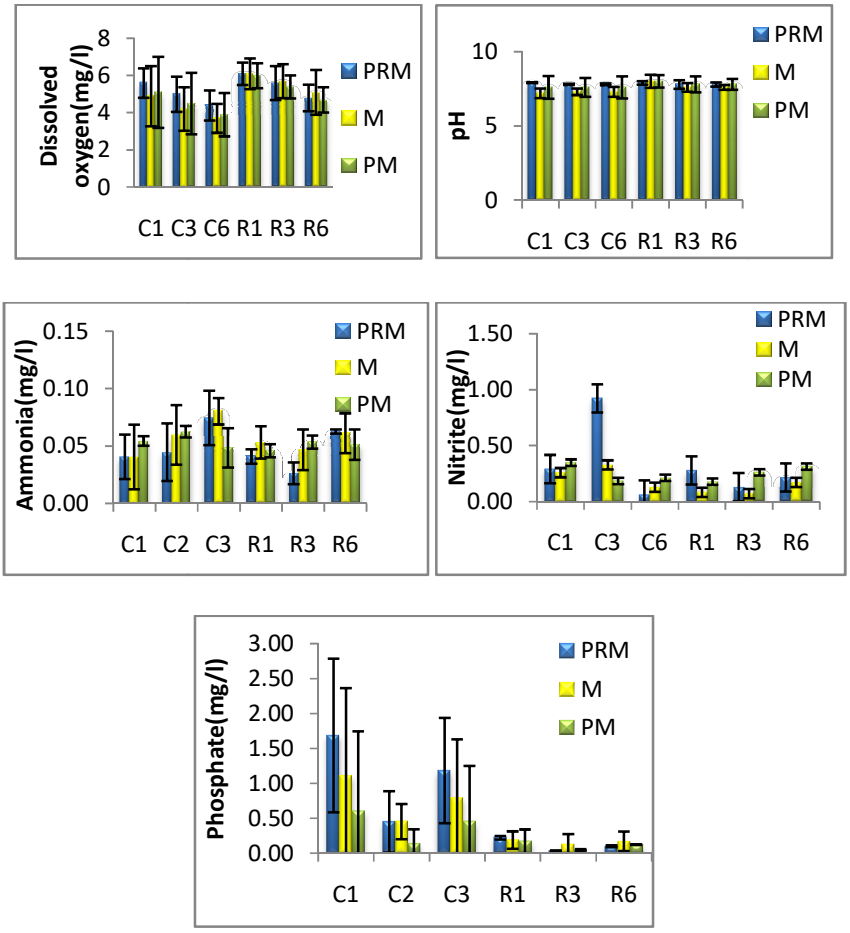


Fig. 4.6.1. Seasonal variation of hydrological parameters at cage and reference experimental test panel. (Error bars indicate the standard deviation)

4.6. 2. Correlation between the fouling organisms and water quality parameters:

The results of two tailed Pearson correlation analyzed in SPSS software were presented.

Table. 4.6. 1.Correlation between the biofoulers and water quality parameters

Correlated parameters	Pearson correlation coefficient (positive correlation)	Significance/p value	Correlated parameters	Pearson correlation coefficient (negative correlation)	Significance/p value
Hydroids*Nitrite	0.598a	0.003	Ascidians*Salinity	-0.651a	0.001
Sea anemones* Nitrite	0.686a	0	Ascidians *DO	-0.630a	0.001
			Hydroids*pH	-0.544a	0.007
			Sea anemones*pH	-0.513b	0.012
			Echinoids*pH	-0.453b	0.03
			Ascidians*pH	-0.433b	0.039

a- Correlation is significant at the 0.01 level (2-tailed).

b- Correlation is significant at the 0.05 level (2-tailed).

4.6.3. Correlation between the fouling communities:

The result of analysis of correlation between the biofouling communities is represented in table 4.6.2.

Table. 4.6. 2.Correlation among the biofoulers

Correlated parameters	Pearson correlation coefficient	Significance/p value
Sponges* Hydroids	0.556a	0.006
Hydroids* Sea anemones	0.752a	0
Hydroids* Echinoids	0.441b	0.035
Sea anemones* Echinoids	0.597a	0.003
Bryozoans* Isopods	0.601a	0.002
Flat worms * Barnacles	0.841a	0
Flat worms* Ascidians	0.584a	0.003
Barnacles * Ascidians	0.624a	0.001
Shrimps* Modiolus	0.938a	0
Shrimps* Ophiuroids	0.941a	0
Scallops * Nudibranchs	0.831a	0
Modiolus* Ophiuroids	0.888a	0
Oysters* Limpets	0.975a	0

a- Correlation is significant at the 0.01 level (2-tailed).

b- Correlation is significant at the 0.05 level (2-tailed).

4.6.4. Relationship between water quality parameters and biofouling community indices:

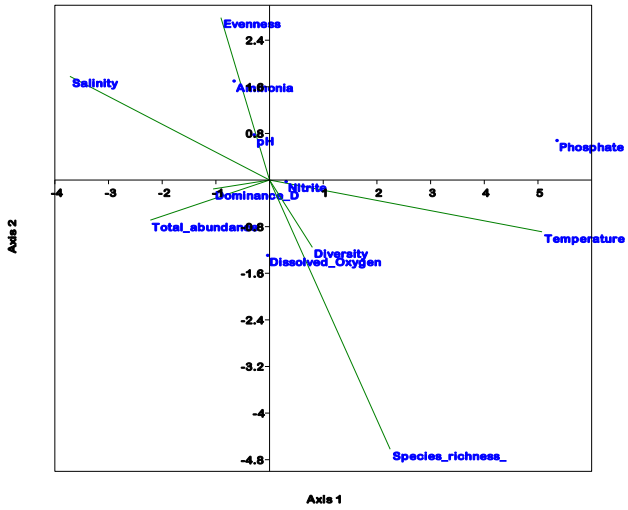


Fig. 4.6.2. CCA of relationship between the water quality parameters and biofouling community indices.

When the temperature, phosphate levels were high the species richness was relatively high. When the salinity and ammonia levels was more, the evenness was relatively more. The species richness was low when the salinity was lower. Diversity was more when dissolved oxygen and nitrite levels were relatively high.

Discussion

Water quality studies of Indian cage culture sites were carried out by Joseph et al. (2010); Philipose et al. (2010; 2012). Philipose et al. (2010), studied the variations of environmental parameters from same sea cage farm of Karwar in the west coast. But literature on relationship between the water quality parameters of finfish cage site and biofouling community indices in west coast is not available.

The results obtained using CCA of relationship between the water quality parameters and biofouling community indices showed that temperature, phosphate levels were positively influencing species richness. Salinity and ammonia levels were influencing the evenness. Low salinity resulted in low species richness. Higher dissolved oxygen and nitrite levels found to lead to higher diversity. According to well known principal of Maguran (1988), biofouler diversity and evenness decreased with

intensification of dominance. In present investigation the dominance of biofoulers was inverse to the evenness and diversity. Similar results were obtained in wooden panel studies of biofoulers in Vishakhapatnam jetty (Pati et al., 2015).

The community compositions of the biofoulers were reported to have significant correlation with the various environmental parameters (Fitridge et al., 2012). Pati et al. (2015) concluded that biofouling community structure was mainly influenced by BOD, salinity, nitrite and phosphate, similar results were obtained from the present study also.

Environmental parameters like temperature and salinity have impact on development and recruitment of biofoulers (Anil et al., 2010). Nasar (2017) while investigating biofoulers assemblage on experimental test panel in two locations, observed that nutrients play important role in development of communities and the biofouling pattern was determined by combined effect of water quality parameters (temperature, ammonia, nitrite and phosphate).

The flatworms and barnacles density were positively correlated (r=0.841, at 0.01significance level), similar positive correlation among the flatworms and barnacle density due to commensalism was observed by Sahu et al. (2015) in the Kalpakkam waters, on the teak wood panels in Bay of Bengal. Assemblage of fouling organisms varied in different depth of the water column. Variation in light penetration, salinity, temperature, dissolved oxygen, pH, water current and physico chemical characteristics were reported as the reasons for the positive correlation. In the present study biofouling is mainly contributed by cnidarians (hydroids) and hydroid showed a significant negative correlation (-0.544) at 0.01 level with pH and showed a significant positive correlation with nitrite (0.598). Ascidians showed a significant negative correlation with salinity i.e. the number of ascidians decreased with the increase in salinity. Salinity, dissolved oxygen, pH exhibited significant negative correlation with ascidians, hydroids, sea anemones, echinoids so as to influencing the biofouling community structure. Similarly the nitrite has showed a significant positive correlation with hydroids and sea anemones influencing the biofouling community structure in the Karwar waters. The similar results have been obtained by Pati et al. (2015) where they concluded that BOD, salinity, nitrite, silicate and phosphate were influencing water quality parameters determining the community structure of biofoulers in Vishakhapatnam harbor.

Most of the biofoulers are having significant positive correlation with other foulers. Sponges showed significant positive correlation with hydroids and echinoderms. Bryozoans have significant positive correlation with isopods, because the isopods were found near the bryozoans in clusters, may be they can take shelter or feed on the bryozoans. Association of bryozoans and other foulers was an important ecological aspect during the developmental sequences of population of fouling communities (Scheer, 1945; WHOI, 1952).

4.7. Influence of environmental parameters on biomass of biofoulers

4.7.1. Correlation between environmental parameters and biofouling biomass

Results of statistical analysis of correlation between the biomass on the experimental test panels and environmental parameters showed that, biomass of biofoulers at 3m. depth panel on the cages have significant negative correlation coefficient with ammonia, -0.639 ($p \leq 0.001$). At 6m. depth panels on cages, fouling biomass has showed significant positive correlation coefficient with phosphate, 0.756 ($p \leq 0.001$). At reference site, on 3m. depth panel, biofouling biomass showed significant negative correlation coefficient with temperature, -698 ($p \leq 0.001$).

4.7.2. Similarity in distribution of biomass of biofoulers in different depth experimental panels of the study sites

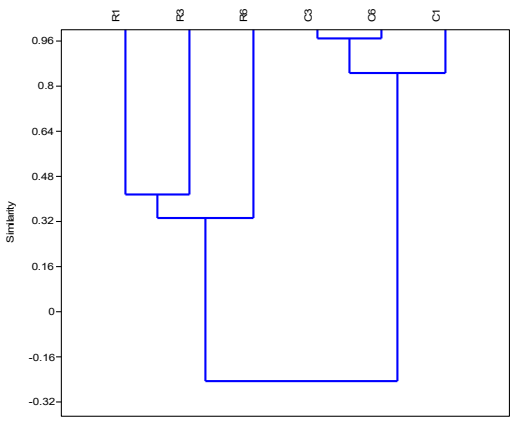


Fig.4.7.1 Dendrogram showing the biomass, cluster analysis (correlation similarity measure) (Concentration ellipse level=95%)

Cluster analysis was done in PAST using correlation (similarity measure) to find the similarity in distribution of biomass of biofoulers in different depth experimental test panels of the study sites. The clusters indicated the existence of completely different sets of clusters in cage and reference site. The cluster analysis of biomass of biofoulers between panels showed clear grouping and significant difference between the two sites (fig 4.7.1).

4.7.3. Similarity analysis of biomass of biofoulers between the months

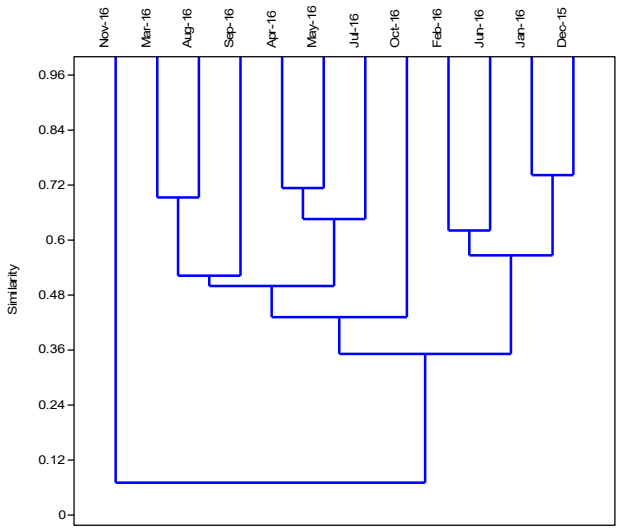


Fig. 4.7.2. Dendrogram showing similarity of biomass composition between the months

The cluster analysis of the observations on biofouling biomass of C1, C3, C6, R1, R3, R6 (experimental test panels) between the months indicated that biomass in November-2016 was different from the other clusters, i.e. The cluster analysis of biomass of biofoulers between the months showed clear grouping between the November-2016 and other months. February-2016 and January-2016 had higher similarity (fig 4.7.2). The one way ANOVA results showed that there was no significant different between the biomass between the panels ($p = 0.697$) but within the months there is significant difference ($p < 0.05$)

Discussion

Higher ranges of ammonia, phosphate and organic carbon have been recorded near the Mediterranean fish farms (LaRosa et al., 2002; Pitta et al., 1999). The bivalves blue mussels were reported to grow faster in nutrient rich waters near to marine cages (Strirling and Okamus, 1995; Cook et al., 2006). In the present study also the higher biomass ranges were constituted by bivalves, green mussels (*P. viridis*) in the cage culture site as reported in the earlier studies. Experiments on marine cage culture site and reference artificial panels conducted in European water (Cook et al., 2006) emphasized higher biofouling biomass of the cage culture site due to higher nutrients availability. The present research findings also support the same.

Study conducted in Arabian Gulf, by Nasar (2017) indicated the salinity, ammonia, phosphate, nitrite and nitrate have strong correlation with mariculture site at Bahrain. Our present results of the experiment also support the study indicating the environmental variables (temperature, salinity, pH, ammonia, nitrite and phosphate) strongly influencing the biomass of the biofoulers at different depths.

The cluster analysis of biomass of biofoulers between the months showed clear grouping between November-16 and other months. This was due to the dominance of the climax community, green mussels covering the experiential panels in November-16 in the cage site. The general studies on biofouling in the coastal waters of Kalpakkam, east coast of India on concrete panels showed considerable biomass variations with season and depth.

Chapter 5: Summary and Conclusion

The study dealt with the composition, distribution, community studies, diversity, biomass, density, succession and correlation with the environmental parameters of biofouling organisms conducted at the fish cage farm in the west-coast of India, in the Arabian Sea at Karwar during 2014-2016.

Total 57 biofouling species belonging to 11 phyla were recorded in the present study. Totally 20 classes, 32 orders and 48 families have been recorded. Phylum

cnidarians formed the major portion of biofouling composition, followed by mollusca and arthropoda. Some biofouling communities were site and location specific and their occurrence varied between the site, depth, between the months and seasons. Sponges, hydroids, bryozoans, polychaete worms, amphipods, barnacles, crabs, isopods, green mussels and *Modiolus* were the common biofouling communities on all the study locations. There was a statistically significant difference between season and the biofouling communities ($P<0.05$).

Seasonal biodiversity indices studies revealed density were high during pre monsoon season on experimental panels and culture net. Seasonal diversity indices of the experimental panels, culture net and the barrels showed that low diversity in deeper panels. Seasonal studies showed that at 6m. depth cage panel, species richness was less during monsoon and post monsoon. The richness was maximum on net during post monsoon with commencement of spat settlement of green mussel. Based on the biomass studies at the cage site, green mussels were the dominant foulers adding to maximum biomass followed by barnacles. Different succession patterns were observed in two sites as well as in different cage structures studied. Hydroids were the first to settle on the long-term panels during the initial month (December) and green mussels formed the climax community in the cage site panel.

Environmental parameters, salinity, temperature, pH, dissolved oxygen and nutrients have the significant contribution on the settlement of the biofoulers. Seasonal studies showed that ammonia levels were low during pre monsoon and high during monsoon. The community compositions of the biofoulers have significant correlation with salinity, Dissolved oxygen, pH and nitrite. Biofouling was mainly contributed by cnidarians (hydroids) and showed a significant negative correlation with pH and significant positive correlation with nitrite. The water quality parameters influence the diversity, fouling community structure and composition of biofoulers.

Conclusion

As biofouling is a major problem in the open sea cage culture practices in Indian waters, affecting the cage accessories, the present study could bring out invaluable information on biofouler diversity and the major biofoulers of the open sea cage culture site of Karwar, west coast water. Total 57 biofouling species belonging to 11 phyla were

recorded in the present study. Phylum cnidarians formed the major portion of biofouling composition, followed by mollusca and arthropoda. Hydroids by number and green mussels by biomass were the major biofoulers of the present study. Hydroids were the major biofouling community contributing to biofouling in the July month, causing damages to the nets. In November, green mussels were the major biofoulers by biomass causing maximum damages to net. Cnidarians being more in density clogged the net, there by disturbing the easy flow of water in and out of the culture nets and the green mussels by increasing the weight of the culture net increases the risk of damage to the net.

Hydroids were the first to settle on the long- term panels during the initial month and green mussels formed the climax community in the panel at cage site. It is the first study in the open sea cage site in Indian waters revealing the pattern of succession of biofouling communities on the experimental test panel, which will help in further research.

Overall it is concluded that, this is a pioneering study in the biofouling research in open sea cage culture in India documenting the 57 biofoulers. This is a first attempt to study diversity and succession pattern of biofoulers on the experimental panels in the cage culture site compared with the reference (control) site.

Environmental parameters have the significant contribution on the settlement of the biofoulers and they influence the diversity, community structure and composition of biofoulers. Biofouling was mainly contributed by cnidarians (hydroids) and showed a significant negative correlation with pH and significant positive correlation with nitrite. Due to higher nutrient availability in the cage culture site the fouling biomass was high, (contributed by *Perna viridis*) compared to the reference site which is proven by correlation studies between the biofouling biomass and the water quality parameters.

Recommendations

With the present information we can aim to inform the fish farmers about temporal occurrence of biofouling communities that cause economic loss during culture period. This data may help us to predict the settlement period of the major biofoulers and properly plan best husbandry practices (Sievers et al., 2014). Information on biofoulers is one of the most important input information in mariculture site selection, which need to

incorporated in suggesting the suitability of the site for cage installation and maintenance. Inventory on befouling organisms prepared from the present study is the first one of that kind from a mariculture site in India which will be of immense help in decision making in cage culture. The studies on influence of environmental parameters on biofouling will help us to assess the future site selection for the cage farming. Since India is in developing stage in cage culture, there is remarkable opportunity for the further development of cage farming in India by incorporating information on biofoulers and also these results opens up much more opportunities to conduct research on biofouling organisms in maricuture especially, how to reduce or avoid biofoulers in space and time. The results of present study will help in initiating further research on biofouling and thereby developing control measures to contain biofouling and to increase the feasibility of cage culture practices.

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Chapter 7: Publications

Published Papers:

1. **A study on ecological succession of macrofouling communities in a sea cage farm in south west coast of India (2021**): **Sonali S. Mhaddolkar**, **A.P. Dineshbabu,** Jayasree Loka, Sujitha Thomus. **International Journal of Life sciences and Pharma research**, 2(2): 159-171.
2. **Impact of water quality parameters on diversity and intensity of biofouling at sea cage farm, Karwar, Karnataka, India (2019)**. **Sonali S. Mhaddolkar**, **A.P. Dineshbabu,** Sujitha Thomas, Jayasree Loka. **International Journal of Life sciences,** 7(4): 655-664
3. **Bio fouling organisms on the experimental panels in cage farm off Karwar, West coast of India (2018). Sonali S. Mhaddolkar**, A.P. Dineshbabu, Jayasree Loka. Lake 2018: **Conference Proceedings on Conservation and Sustainable Management of Riverine Ecosystems [The 11th biennial lake conference] 22-24 November 2018**:151-160.
4. **Experimental studies on Macro fouling communities on net panels at marine cage farm of Karwar, India (2017)**: **Sonali S Mhaddolkar**, Jayasree Loka, K.K. Philipose and A.P. Dineshbabu, published in **International Journal of Fisheries and Aquatic Studies, 5(2): 184-187.**

Manuscripts under review:

- **Diversity and distribution of biofoulers observed in marine cage farm At Karwar, West coast of India**. **Sonali S. Mhaddolkar,** A. P. Dineshbabu, Sujitha Thomas, Jayasree Loka sent for **Journal of Marine Biological Association of India** on 21.01.2020.

- **Experiment to evaluate impact of environmental parameters on the biofouling biomass in open sea cage culture site**. **Sonali S. Mhaddolkar**, A.P.Dineshbabu submitted to **International Journal of life Sciences and pharm research.**

Paper Presentation in National seminars

1. **Presented poster presentation in XIIIth Agriculture Science congress (2017):** 21-24 February 2017 at Bangalore, Micro and macro fouling communities on the experimental net panels in marine cage farm of Karwar, West coast of India.

2. **Presented paper in Lake Conference (2018):** Conference on Conservation and Sustainable Management of Riverine Ecosystems [The 11th biennial lake conference] on 22-24 November 2018. Bio fouling organisms on the experimental panels in cage farm off Karwar, West coast of India.

3. **National workshop on Marine biodiversity and climate change (2014):** held at Karwar RC of CMFRI on 21-20 May 2014. Presented a paper "Biodiversity studies in the marine cage farm off Karwar". Sonali S. Mhaddolkar, N. G. Vaidya, Jayasree Loka, Krupesh Sharma, K. K. Philipose.

4. **Poster presented in 5th International Symposium on Cage Aquaculture in Asia (2015):** Organized by Asian Fisheries Society at Central marine Fisheries Research Institute, Kochi. Presented Biodiversity of macro fouling organisms in a marine cage farm at Karwar, west coast of India, during 25-28 November 2015.

5. **Poster presentation in 3rd national seminar on Current trends in Life Sciences, NSCTLS (2021):** on "Experiment to evaluate impact of environmental parameters on the biofouling biomass in open sea cage culture site. Sonali S.Mhaddolkar, A.P.Dineshbabu"on 29.01.2021,organized by Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior in association with NASI (Bhopal Chapter) and Society of Life Sciences.

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International Journal of Life science and Pharma Research

Research Article

Environment science for better human health

A Study on Ecological Succession of Macrofouling Communities in Sea Cage Farm in South-West Coast of India

Sonali S. Mhaddolkar[1,2*], A.P. Dineshbabu[3], Jayasree Loka[1] and Sujitha Thomus[3]

[1]*Karwar Research Centre of Central Marine Fisheries Research Institute, P.B.No.5, Karwar, Karnataka, India.*

[2] *Department of Biosciences, Mangalore University, Konaje ,Karnataka, India.*

[3]*Mangalore Research Centre of Central Marine Fisheries Research Institute, PB No.244, Hoige Bazar, Mangalore, Karnataka, India.*

Abstract: Development of aquaculture facilities like cages has led to rise in submerged structures which provide ample substratum to biofoulers which could greatly interfere with culture operations. An attempt was made to study the biofouling communities and succession of macro foulers on the cage culture net installed in the open sea. The main objective of the present study is to reveal the succession pattern of the biofouling communities on the panels of cage culture sites to find out seasonal settlement pattern, Dominant species and Climax community. A long-term study on the succession pattern of the cage farm experimental-net-panels revealed results as Hydroids-Gastropods-Hydroids-Barnacles-*Modiolus*-Green mussels. Hydroids were initial communities on the net panels and green mussels (*Perna viridis*) formed the climax community, also dominating on the cage culture nets. Different succession patterns were observed in two sites as well as in culture nets studied. The net panels of the cage were loaded with hydroids in the initial months and the peak fouling was during May. So frequent net cleaning was required during summer and during the spat settlement period of green mussel (September, October and November). *Modiolus* settlement during February month on the culture nets can be avoided by net exchange immediately after spat fall in this month. This attempt was made to study the ecological succession on the panels, in cages installed in Karwar, which is the first attempt, since the open sea cage culture was initiated in India. Looking at vast opportunities for further development in biofouling research, the aim of this investigations was to obtain the baseline information about the ecological succession pattern of biofouling organisms in fish cage sites. More research on biofouling in mariculture is essential to ensure the profitability of the aquaculture operations with environmental safety measures as a prime criteria.

Keywords: macro fouling communities, succession, climax community, panels, sea cage farm.

***Corresponding Author**

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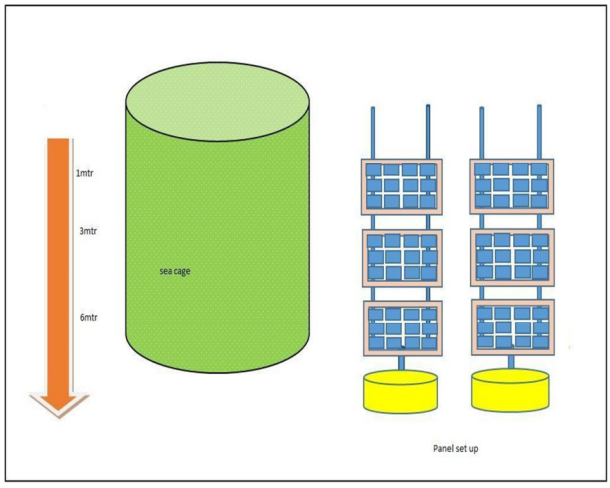
1. INTRODUCTION

Development of aquaculture facilities like cages has led a sore in submerged cage-structures like nets, floats, ropes which provide ample substratum to the biofoulers, greatly interfering culture operations.[1] The assemblage and development of biofouling communities is a typical exemplar for succession process.[2] Many studies were undertaken to understand the spatio temporal succession patterns of the biofoulers and to ascertain the period in which interventions are required to reduce the loss of aquaculture materials due to biofouling.[3,4] Panels were used widely to study the biofouling and several researchers have worked on the succession involving size, duration, location, season, months for studying the biofouling on different structures mainly ships hulls, water exchange pipes, jetties, buoys, mariculture structures and other submerged surfaces.[5,6] Research are being carried out in biofouling on mariculture structures worldwide.[7,8,5,9] The ecological succession of biofoulers is a very complex process and development pattern found on the suspended cage structure may vary from the natural sea bed and hard substratum.[10] Formation of biofouling community is site specific[11] and their development with respect to time is prerequisite for the marine structures and for developing cleaning practices.[12] Ecological process of biofouling community-development may take a day or weeks[4] and biofouling communities varies with time and space but the major variations were the seasonal variations.[10,13,14] Duration of substrate immersion is an important aspect to be considered for studying the assemblage of biofouling in succession experiments.[1] Along with the other factors predation was a major factor affecting the succession of the fouling communities.[10] Severity of settlement varied on different substrata based on the choice of settlement of planktonic larval-forms. [15]Succession studies at different depth were by Dziubińska and Szaniawska.[2] The attaching foulers forms formed the bigger components but the free-living forms were also found in the community of biofoulers.[16] Succession is a composite process in which simple organization level changing to compound community form.[10] Comprehensive studies on marine biofouling process were carried out by Railkin.[17] Pioneering study in biofouling along the Indian coast were conducted on different structures such as experimental panels, ship halls, jetties etc.[16,18,19] Many research works were carried on the fouling community structure.[5,11,20-22] Literature review about succession and climax communities of biofouling in general (other than mariculture facilities) is available along the Indian coast[11] ,but very less literature is available on the ecological succession of biofouling on the panels in marine culture sites, along the coasts of India. This attempt was made to study the biofouling and ecological succession on the panels, in cages installed in Karwar, which is the first attempt on open sea cages in India.

2. MATERIAL AND METHODS

In marine cages installed in the Arabian Sea at Karwar, Karnataka (N 14°48.406', E 074°06.664'), so far six species of finfishes (*Lates calcarifer, Rachycentron canadum, Lutjanus argentimaculatus, L.johnii, Trachinotus blochii, Acanthopagrus latus)* and one shellfish species (*Perna viridis)* are being successfully farmed. For experimental studies two sites were chosen so as to compare between the cage and the reference. The experimental panels were installed in marine farm and the reference site during the period 2015 to 2016. Annual Panel setups were made with 12, HDPE net panels (100mm^2) of mesh size 22 mm fixed to 19mm dia half inch PVC pipe frame. They were tied by using 4mm nylon rope in the water column adjacent to the cages in the culture site. (fig.1)These panels were anchored with 5 kg weight to stay in vertical position in the water column. Reference site is 500m away from the cage site where the panels were placed using the barrel and the anchor. Every month three panels (from 1m, 3m, 6m depth) from each site were brought to the laboratory for analysis. Monthly, seasonal (exchange) data of culture net are collected for further analysis.



The orange arrow indicates the 3depths, sea cage (represented by Green cylinder), panel set up (orange colour rectangles)with net panels (blue colour)and weight (yellow colour)

Fig 1. Diagrammatic representation of field setup at cage site

2.1 Laboratory studies

The net panels were brought to the laboratory separately in the plastic trough with sea water. The fouling organisms were washed with sterile sea water and sieved in 200-micron sieve.[23] The fouling samples were preserved in 5% formaldehyde for further identification. Smaller fouling organisms were observed under AXIO, Zeiss (Scope-A1) microscope (5x magnifications). Taxonomic Identifications were done using identification keys.[24-26] Density

(number/10cm^2), total length, percentage of major macro fouling organisms were studied using digital vernier caliper and photographic images. The identified foulers were reclassified to different groups (community).

3. STATISTICAL ANALYSIS

The data collected was analyzed statistically using SPSS16.0, PRIMER 0.5 and XLSTAT2016 softwares. Average data of the panels from three depths of each site was taken for the analysis and comparison between the sites.

4. RESULTS

4.1 Succession of biofouling on long term panels

Hydroids were the initial settlers dominating from December to May on the experimental panels of cage and reference sites. June 16, they totally vanished from both the sites. The density of hydroids ranged from85 no/10cm^2 in January-16 to1433 no/10cm^2 in July-16 in the cage site whereas, 180no/10cm^2 in January to 1733no/10cm^2 August in the reference site. In June gastropods dominated both in cage sites with 16no/10cm^2 and reference sites with33 no/10 cm^2. During July and August, hydroids dominated. Oysters dominated in August (57no/10cm^2) in reference site where as in September barnacles dominated with 41no/10cm^{2}in cage site and green mussels dominated (29no/10cm^2) in reference site. In October *Modiolus* dominated in both the sites with 547no/10cm^2 and during November green mussel was most dominating (188no/10cm^2) group in cage site and Amphipods were the dominant communities (43 no/10cm^2) in reference site. Fig. 2 & 3 represents dominant fouling organisms on long term panels of cage and reference site.

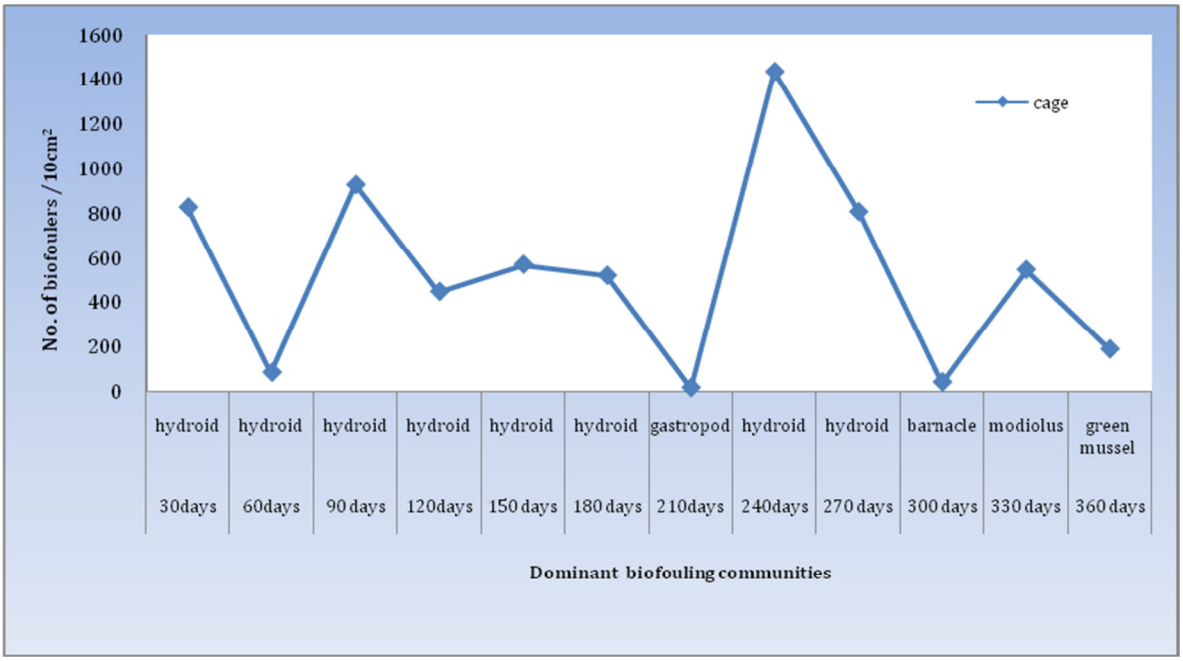


Fig 2. Dominant biofouling communities of cage site during the immersion period (30 to 360 days)

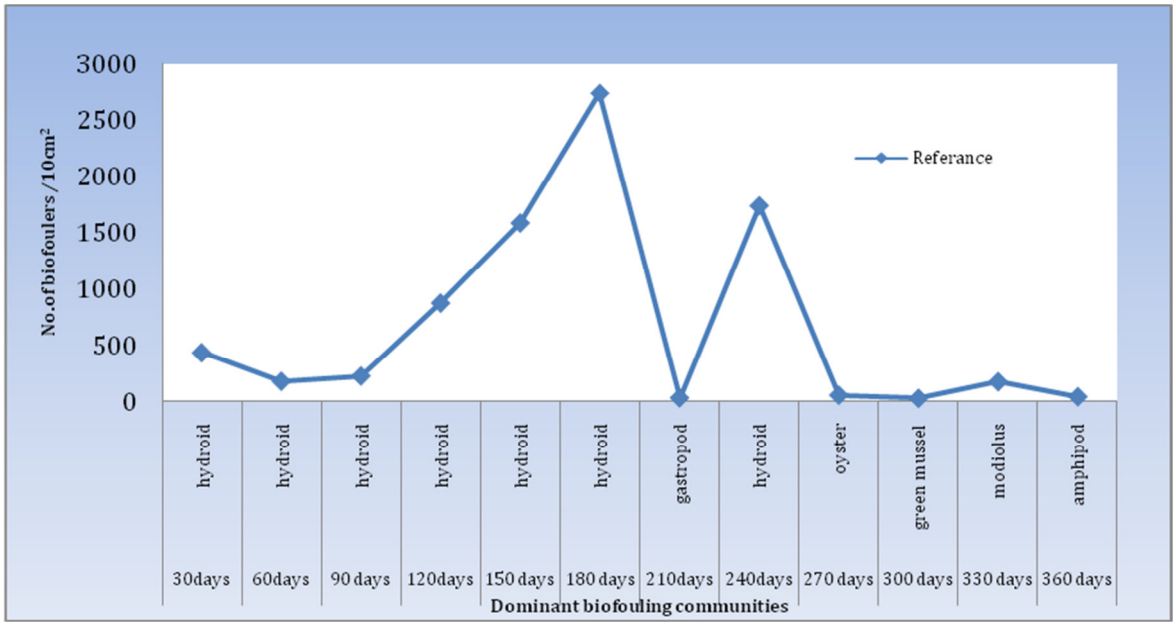


Fig 3. Dominant biofouling communities of reference site during the immersion period (30to360 days)

The pioneering biofouling communities on the panels was composed of hydroids, barnacles, green mussels in December-15(30 days) panel in cage site where as in reference site along with hydroids, polychaete worms, amphipods, barnacles, crabs, shrimps, Isopods, green mussels, *Modiolus,* oysters, were the fouling communities got settled. In January-16(60 days) panel bryozoans, crabs, shrimps and *Modiolus* started appearing on the cage panels where as in the reference site bryozoans started appearing on the panel and polychaete worms, amphipods, shrimps, isopods started disappearing from the panel. In February-16 (90days) amphipods, isopods, gastropods, oysters got introduced on the panel. Considerable density of isopods and slight increase in the bryozoan number were observed during this period but Crabs and shrimps disappeared and decrease in the barnacle number also were observed in cage sites, whereas in reference site barnacle density decreased. Crabs, green mussels, *Modiolus*, oyster density started decreasing and they vanished towards the end of this period. Sponges, flatworms, scallops, nudibranchs appeared for the first time on the panel. Polychaete worms, amphipods, Isopods, reappeared during this period. March-16 (120 days) panel, sponges, polychaete worms, echinoids which were not present in previous months were observed during this month in cage site but in reference site, sponge, hydroids, sea anemones, bryozoans, flatworms, polychaete worms, amphipods, Isopods showed an increase in trend. Sea anemones, gastropods, ascidians appeared for the first time on the panel. Scallops and nudibranch disappeared. During April-16 (150 days) there was a slight increase in the sponge density. Crabs disappeared in the cage site but in the reference site, the green mussels, sponge, amphipod, hydroid, sea anemone, barnacle, crab number has increased than the previous period. Nudibranch which disappeared in the previous period has appeared, whereas gastropods and oysters disappeared in this period. In May-16 (180 days) Sea anemones and ophiuroids settled for the first time. The communities of crabs, green mussels, oyster, reappeared which were absent in the previous month in cage site but in reference site Sponge, bryozoans, polychaete worms, Amphipods, crabs, Isopod, green mussel showed a decreasing trend, flatworms, nudibranch and ascidians disappeared during this period. Shrimp and ophiuroids reappeared during this period. June-16 (210 days) most of the communities like hydroids, sea anemones, bryozoans, barnacles, isopods, oysters, ophiuroids disappeared and Ascidians got introduced for the first time and Echinoids reappeared on the cage panel. In reference, most of the fouling communities disappeared during this period. Polychaete worms, amphipods, barnacles, crabs, gastropods, green mussels, *Modiolus* were present. Hydroids showed a complete decline. Sponges, hydroids, sea anemones, bryozoans, shrimps, Isopods, oysters, echinoids, ophiuroids which were present in the previous period were completely disappeared. *Modiolus* appeared during this period. In July-16 (240 days), Hydroids again reappeared along with Bryozoans and barnacles. Amphipods, gastropods, Ascidians which were present in the previous month disappeared from the panels. In the reference site sponges, hydroids, sea anemones, bryozoans, barnacles, crabs, green mussels, *Modiolus*, echinoids and ascidians were the fouling communities on the panel. The polychaete, amphipods, gastropods which were present in the previous period, disappeared. Most of the communities which disappeared during the previous duration have started appearing again like sponges, hydroids, sea anemones, bryozoans, echinoid, ascidians. By August-16 (270 days), flat worms got introduced for the first time. Amphipods *Modiolus*, Ascidians, reappeared in the panel, whereas in the reference panel Sponges polychaete worms, amphipods, barnacles, crabs, green mussels, oyster limpets were present on the panel. The communities which were present during the previous period like hydroids, sea anemones, bryozoans, *Modiolus* disappeared. Polychaete worms, amphipods, oysters and limpets, reappeared on the panel. Oysters were the dominating community (57no/10cm^2). Barnacles were the next dominating community. By September-16 (310days), oysters reappeared on the panel. Flatworms, *Modiolus*, ascidians which were lesser in number during the previous month were completely vanished whereas in the reference site, polychaete worms, barnacles, crabs, Isopods, green mussels, *Modiolus* and oysters were the communities which appeared on the panel. Oysters which were dominant during the previous period started decreasing. Sponge, amphipod, limpets were completely declined. Isopod and *Modiolus* reappeared on the panel. October-16 (340 days) highest density was of *Modiolus*. Bryozoans and oysters disappeared in this month where as in reference polychaete worms, Barnacle, crabs, Isopods, green mussels, modiolus were present on the panel. Density of barnacle, isopod, *Modiolus* showed an increasing trend. Oyster density declined. By November-16 (360 days), the panels were fully covered by green mussels (100%) and the community reached its climax stage. Whereas hydroids, sea anemones, polychaete worms, amphipods, crabs, gastropods, and *Modiolus* were the communities observed in the reference panel and Amphipods were the dominant communities (43 no/10cm^2).

4.2 Seasonal succession of the long-term panels

In the present case the long term cumulative panels were studied up to360 day (1year). The settlement pattern revealed Hydroids as prominent communities in all the seasons both in culture site and reference site (fig.2&3). Cage sites the high settlement of hydroid, barnacles, *Modiolus* and green mussels was observed, along with the gastropods. Where as in the reference , where there was no culture activities, hydroids, oysters, anemones, *Modiolus* have settled on the panels along with gastropods and amphipods on the panel. Other than these major fouling organisms, sponges, sea anemones, bryozoans, flatworms, polychaete worms, pycnogonida, crabs, shrimps, Isopods, scallops, nudibranchs, limpets, echinoids, ophiuroids and ascidians were also formed succession sequences as a minor biofouling species. Seasonal succession patterns on the long term studies of cage and reference were presented in the Fig.10.

4.3 Peak settlement period for the major fouling communities on long term panels

Hydroids: peak settlement month is July for the culture site and for reference site May
Modiolus: peak month is October both for cage and reference site
Barnacles: peak month is August both for cage and reference
Green mussels: peak month is November in cage and April in reference
Isopods: peak month is February in cage and March in reference
Polychaete worms: peak month is May in cage site and June in reference

Bryozoans: peak months are September and March for cage and reference.

4.4 *Seasonal fouling on the culture net*

Seasonal settlement pattern (fig.6) and succession pattern biofouling on the culture net (Fig.11) revealed the hydroid and algal dominance in short term fouling studies. Total 22 fouling communities were present on the net viz. algae, sponge, hydroids, sea anemones, bryozoans, flatworms, polychaete worms, amphipods, pycnogonida, barnacles, crabs, shrimps, Isopods, gastropods, green mussels, *Modiolus*, nudibranch, oysters, limpets, echinoids, ophiuroids, and ascidians. During Pre-monsoon season algae were the dominating community with 40.85% followed by hydroids 34.98% and modiolus by 10.89%.The fouling communities like algae, hydroids, sea anemones, bryozoans, flatworms, polychaete worms, amphipods, pycnogonida, barnacles, crabs, shrimps, Isopods, gastropods, green mussels, *Modiolus*, nudibranch, oysters, echinoids and ascidians appeared in this season on the culture net. During Monsoon season hydroids dominated on the net 50.69% followed by algae 16.50%, *Modiolus* 9.31%. Sea anemones, pycnogonida, shrimps, gastropods, nudibranch and ascidians, which were present in the pre monsoon disappeared in this season. Sponges and limpets appeared in this season. Hydroids showed an increasing trend whereas algae showed a decreasing trend. *Modiolus* showed a decline during monsoon. Algae dominated during Post-monsoon season, with 37.70% followed by *Modiolus* 29.18% and hydroids 16.87%. Algae showed an increasing trend and hydroids showed a decreasing trend. *Modiolus* also showed an increasing trend during post monsoon. Flat worms and ascidians which were present in the monsoon are absent in this season.

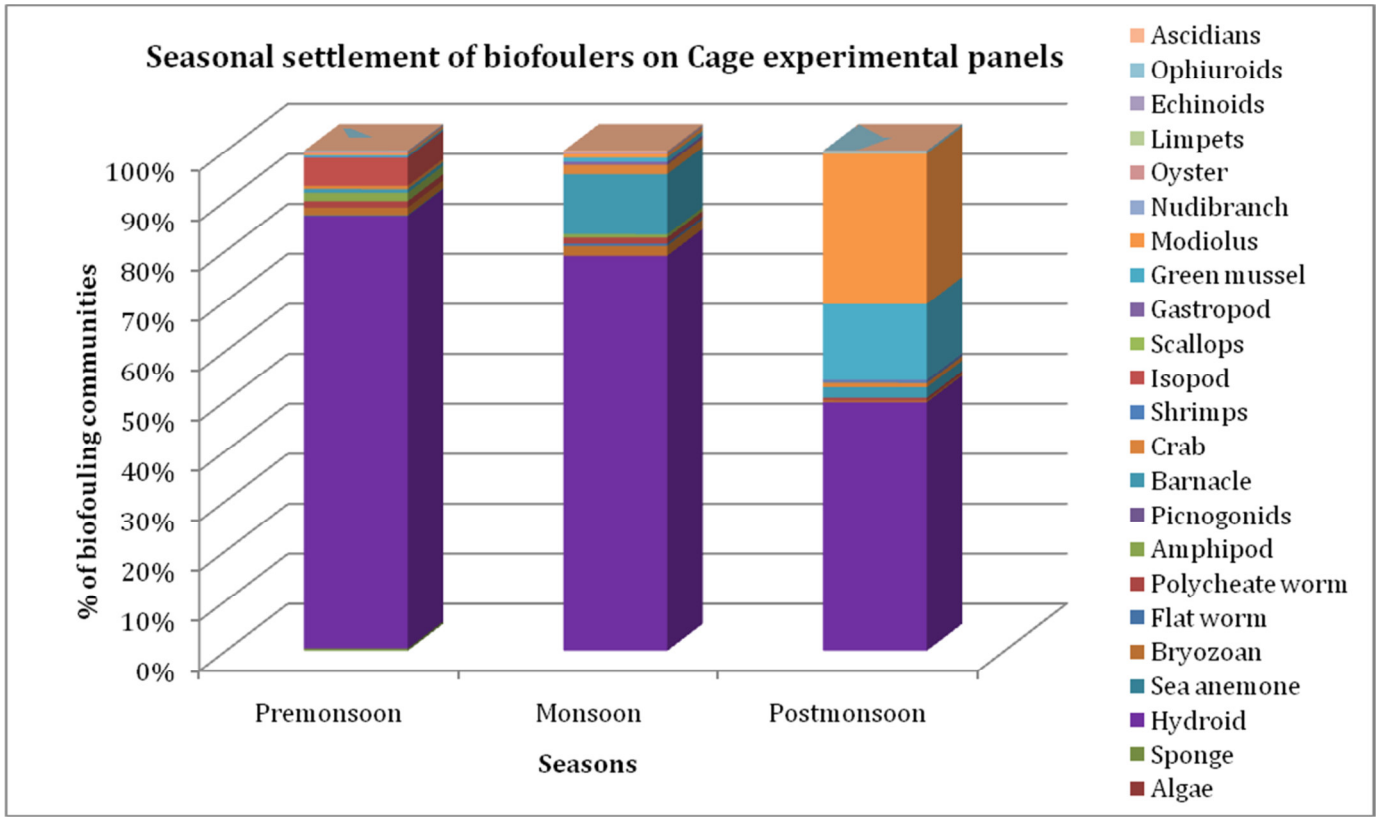


Fig 4. Seasonal settlement pattern of Cage panel

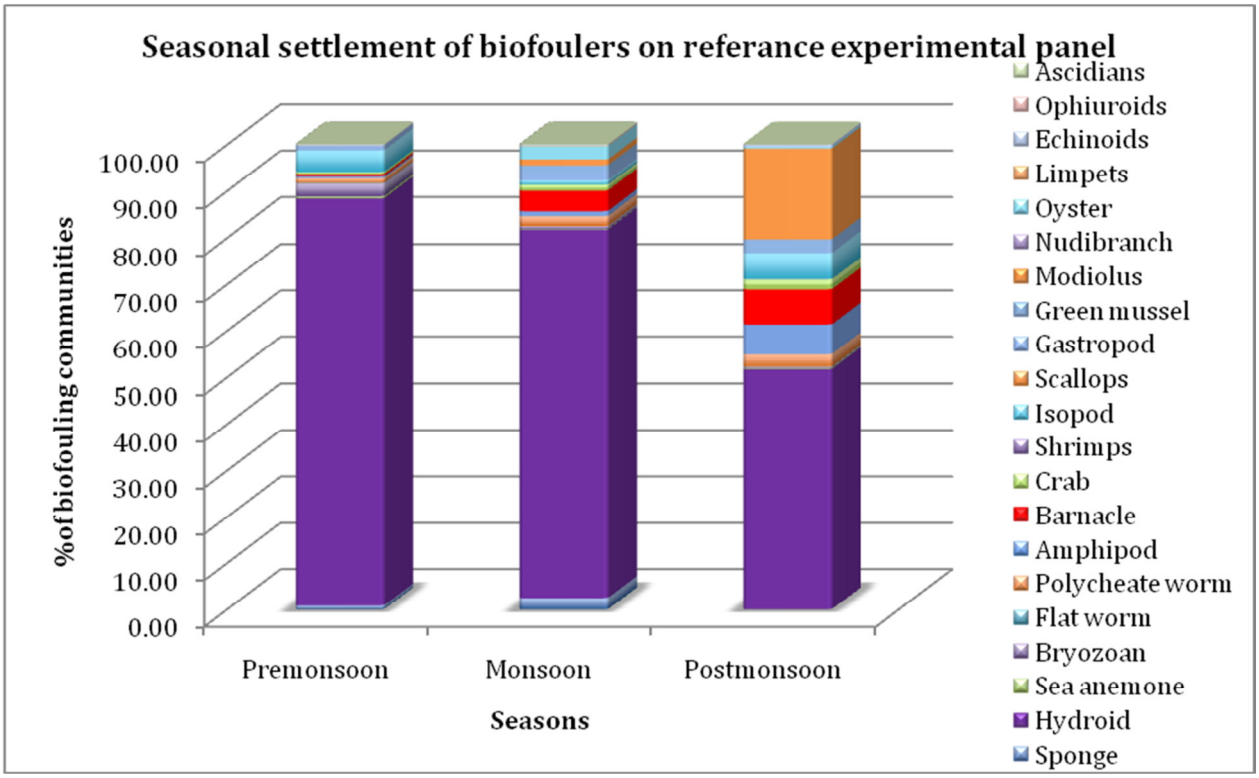


Fig 5. Seasonal settlement pattern of reference panel

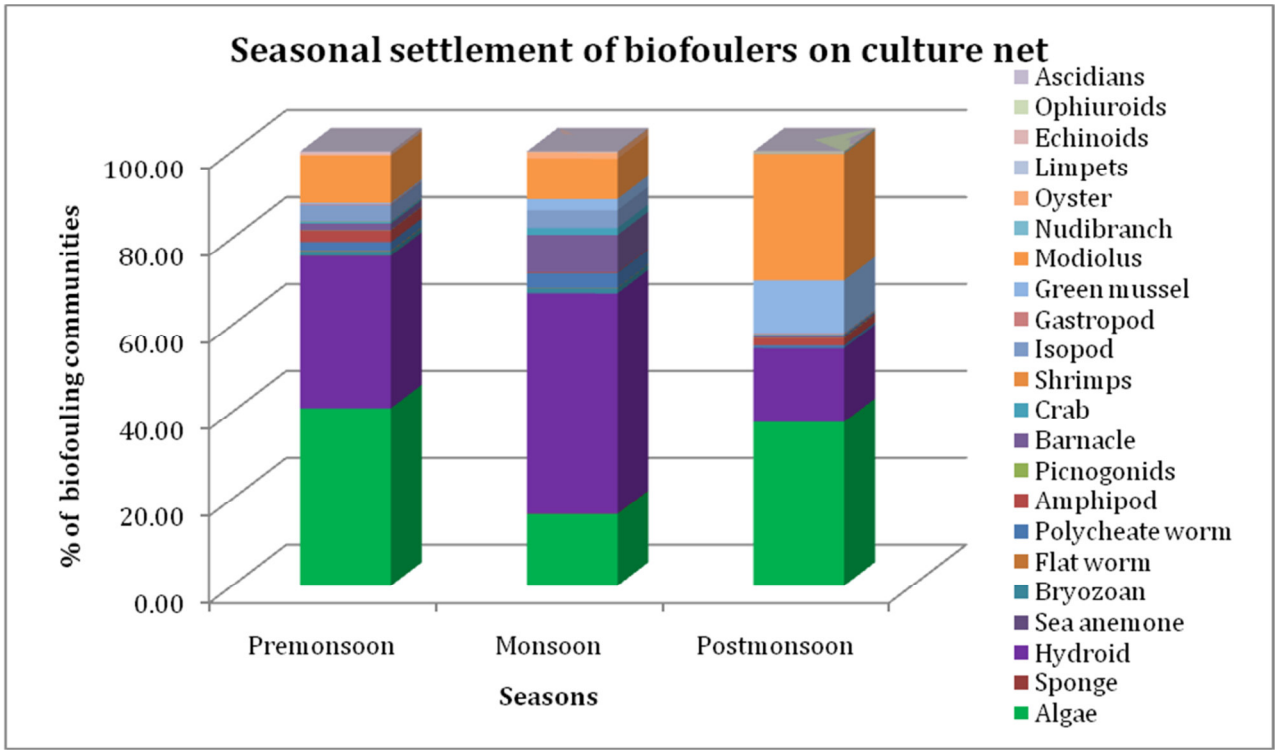


Fig 6. Seasonal settlement pattern of culture net

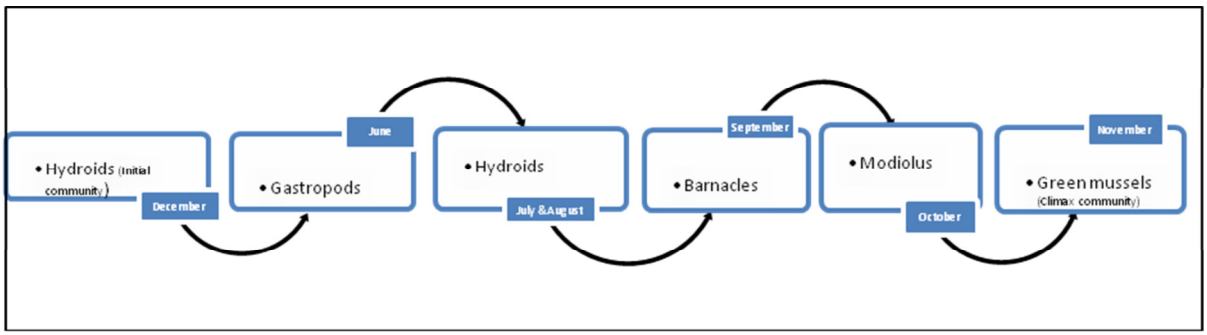


Fig 7. Month wise succession pattern on the long term panels of cage site

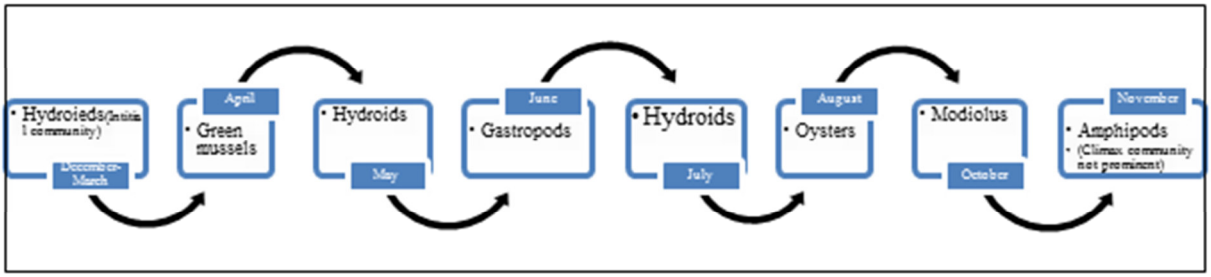


Fig 8. Month wise succession pattern on the long term panels of Reference site

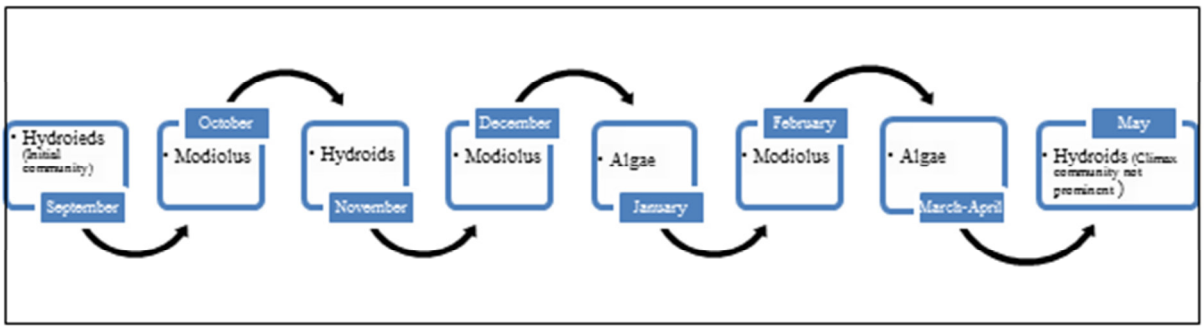


Fig 9. Month wise succession pattern on the Short term cage culture net

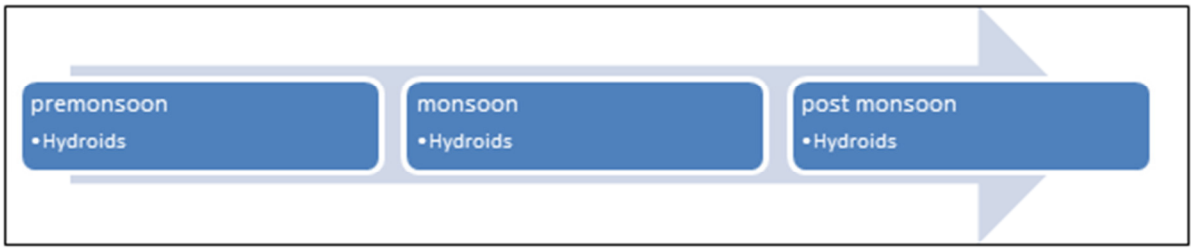


Fig 10 . Seasonal succession pattern on the long term panels (cage site and reference)

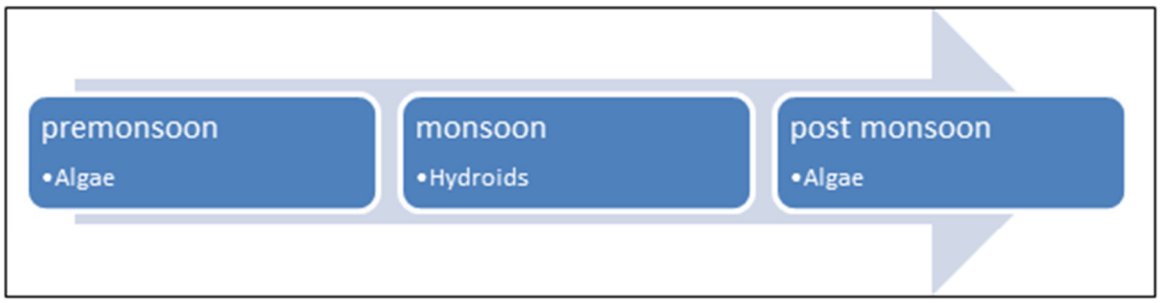


Fig 11 . Seasonal succession pattern on the short term cage culture net

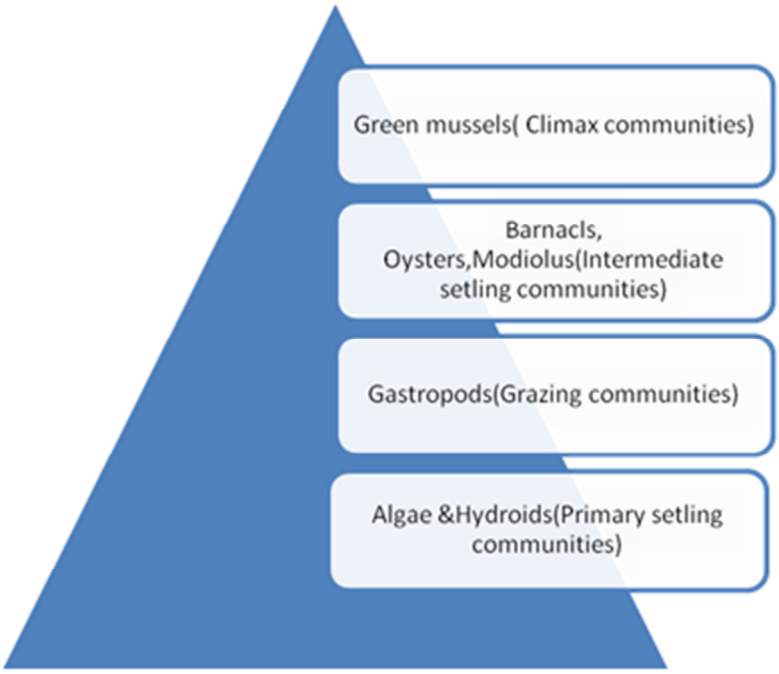


Fig 12. A model of overall macro fouling of cage farm of Karwar, India

4.5 Climax community

Green mussels (*Perna viridis*) were the dominant fouling community forming the climax in the cage site on the net panels after 12 months of immersion. But in the reference site panels and the other structures the climax community is not so prominent. Overall macrofouling in the cage culture site is presented (fig.12).

5. DISCUSSION

This present study can be compared with the biofouling studies in bivalve aquaculture (oyster culture) where in initial colonies included hydroids, bryozoans, sponges, ascidians, polychaetes, bivalves, barnacles and algae.[27,28,29] In bivalve aquaculture practices the primary colonies enables the attachment of groups like crustaceans, polychaete worms or echinoderms and secondary colonization occurred after a month or few month.[27,30] In the present study, hydroids and barnacles were appeared in the initial month in the cage panel whereas hydroids, polychaete worms, amphipods, barnacles, crabs, shrimps, isopods, green mussels, *Modiolus* and oysters appeared in the reference panel. The recruitment, settlement complexities could be the reason for these differences in marine invertebrates.[27,31] Similar reasons could be attributed in the case of fin fish aquaculture cage panel and the reference panel. The biofouling succession as well as colonization patterns differs with climatic zones, as in tropical zones constant settlements around all the months, whereas at fixed intervals in temperate zone.[4,27] The local surveys are necessary as the colony patterns of biofoulers differ along the farming area.[27] Literature on succession studies of biofoulers is available on marine cages of Gulf of Maine, United States of America.[10] The basic information on fouler diversity, composition and succession are required to control the foulers and estimate the fouling potency of that locality. [5,32] Sahu et al.[11] suggested low salinity and high turbidity favorable for barnacles which settled year around.

5.1 Seasonal succession pattern

In the seasonal succession pattern of biofoulers settlement of short-term studies are different from long term studies, similar observations were made by Sahu et al.[12] During monsoon low fouling diversity and density due to low salinity and low temperature.[12] On long term panels, peak settlement was during Mayand in hydroids highest total density was in November (green mussels) in a cage with experimental panels. As far as overall period is concerned, in the May , fouling abundance was more on the cage nets. Cage panel succession was represented in fig. 7. Hydroids were the first to settle on the long-term panels during the initial month (December) along with barnacles, followed by the gastropods in June. During this succession period, barnacles, bryozoans and other fouler were found to colonies on the panel. But the hydroids population was not much affected by secondary fouling communities' settlements. But during June hydroids totally vanished and gastropods were the dominant fouling group along with polychaete worms, ascidians and other species. Again, in July hydroids reappeared and dominated the panels till August. In August barnacles dominated over the hydroids. In October *Modiolus* started dominating, along with them green mussel settlement was also started increasing. Green mussels which started their appearance in May started increasing steadily from August onwards and during November it reached the highest. Total panel was covered by green mussels forming the climax community in the cage site (fig.13). Reference panel succession was represented in fig. 8. In reference site, the hydroids were the first settlers followed by the gastropods, oysters were settled in August, green mussels in June and September, *Modiolus* in October and amphipods in November. The climax community was not so prominent, since it is open waters the grazing and predation may be the factor which is affecting the dominancy and the climax communities of biofoulers.[12] Some Biofoulers showed significance between the seasons, influencing the biofouling community as a whole (table1&2).

Table 1. ANOVA results for site and biofoulers

ANOVA Table							
			Sum of Squares		F		
Sponge * site	Between Groups	(Combined)	314.618	1	314.618	2.739	0.113
	Within Groups		2412.155	21	114.865		
	Total		2726.773	22			
Hydroid * site	Between Groups	(Combined)	116168.775	1	116168.775	0.218	0.645
	Within Groups		11170000	21	531766.432		
	Total		11280000	22			
Sea Anemones * site	Between Groups	(Combined)	35.077	1	35.077	1.927	0.18
	Within Groups		382.324	21	18.206		
	Total		417.401	22			
Bryozoans * site	Between Groups	(Combined)	235.929	1	235.929	0.614	0.442
	Within Groups		8067.617	21	384.172		
	Total		8303.546	22			
Flatworm * site	Between Groups	(Combined)	1.886	1	1.886	0.196	0.662
	Within Groups		201.718	21	9.606		
	Total		203.604	22			
Polychaete worms * site	Between Groups	(Combined)	132.536	1	132.536	2.124	0.16
	Within Groups		1310.324	21	62.396		
	Total		1442.86	22			
Amphipod * site	Between Groups	(Combined)	118.841	1	118.841	0.856	0.365
	Within Groups		2916.367	21	138.875		
	Total		3035.208	22			
Barnacles * site	Between Groups	(Combined)	1731.834	1	1731.834	0.448	0.51
	Within Groups		81148.533	21	3864.216		
	Total		82880.367	22			
Crab * site	Between Groups	(Combined)	1.235	1	1.235	0.029	0.867
	Within Groups		896.672	21	42.699		
	Total		897.907	22			
Shrimps * site	Between Groups	(Combined)	0.443	1	0.443	0.75	0.396
	Within Groups		12.407	21	0.591		
	Total		12.85	22			
Isopod * site	Between Groups	(Combined)	1649.751	1	1649.751	1.043	0.319
	Within Groups		33231.061	21	1582.431		
	Total		34880.812	22			
Scallops * site	Between Groups	(Combined)	0.018	1	0.018	0.913	0.35
	Within Groups		0.407	21	0.019		
	Total		0.425	22			
Gastropod * site	Between Groups	(Combined)	8.432	1	8.432	0.142	0.71
	Within Groups		1250.505	21	59.548		
	Total		1258.937	22			
Green mussels * site	Between Groups	(Combined)	2142.918	1	2142.918	1.197	0.286
	Within Groups		37589.468	21	1789.975		
	Total		39732.386	22			
Modiolus * site	Between Groups	(Combined)	5436.515	1	5436.515	0.386	0.541
	Within Groups		295390.963	21	14066.236		
	Total		300827.478	22			
Nudibranchs * site	Between Groups	(Combined)	4.822	1	4.822	1.906	0.182
	Within Groups		53.139	21	2.53		
	Total		57.961	22			
Oyster * site	Between Groups	(Combined)	140.451	1	140.451	1.009	0.327
	Within Groups		2922.351	21	139.16		
	Total		3062.802	22			
Limpets * site	Between Groups	(Combined)	0.443	1	0.443	0.913	0.35
	Within Groups		10.185	21	0.485		
	Total		10.628	22			
Echinoids * site	Between Groups	(Combined)	1.321	1	1.321	0.274	0.606
	Within Groups		101.094	21	4.814		
	Total		102.415	22			
Ophiuroids * site	Between Groups	(Combined)	7.677	1	7.677	1.691	0.208
	Within Groups		95.357	21	4.541		
	Total		103.034	22			

Ascidians * site	Between Groups	(Combined)	1.583	1	1.583	1.807	0.193
	Within Groups		18.397	21	0.876		
	Total		19.981	22			

Table 2. ANOVA results for season and biofoulers

ANOVA Table

			Sum of Squares		F		
Sponge * season	Between Groups	(Combined)	257.717	2	128.859	1.044	0.371
	Within Groups		2469.056	20	123.453		
	Total		2726.773	22			
Hydroid * season	Between Groups	(Combined)	2305338.653	2	1152669.326	2.568	0.102
	Within Groups		8977925.192	20	448896.26		
	Total		11280000	22			
Sea anemones * season	Between Groups	(Combined)	59.933	2	29.966	1.677	0.212
	Within Groups		357.468	20	17.873		
	Total		417.401	22			
Bryozoans * season	Between Groups	(Combined)	2692.3	2	1346.15	4.798	0.02
	Within Groups		5611.246	20	280.562		
	Total		8303.546	22			
Flatworm * season	Between Groups	(Combined)	10.396	2	5.198	0.538	0.592
	Within Groups		193.208	20	9.66		
	Total		203.604	22			
Polychaete worms* season	Between Groups	(Combined)	165.239	2	82.619	1.293	0.296
	Within Groups		1277.621	20	63.881		
	Total		1442.86	22			
Amphipod * season	Between Groups	(Combined)	136.882	2	68.441	0.472	0.63
	Within Groups		2898.325	20	144.916		
	Total		3035.208	22			
Barnacles * season	Between Groups	(Combined)	10618.889	2	5309.444	1.47	0.254
	Within Groups		72261.478	20	3613.074		
	Total		82880.367	22			
Crab * season	Between Groups	(Combined)	87.148	2	43.574	1.075	0.36
	Within Groups		810.759	20	40.538		
	Total		897.907	22			
Shrimps * season	Between Groups	(Combined)	1.483	2	0.742	1.305	0.293
	Within Groups		11.367	20	0.568		
	Total		12.85	22			
Isopod * season	Between Groups	(Combined)	13253.478	2	6626.739	6.128	0.008
	Within Groups		21627.333	20	1081.367		
	Total		34880.812	22			
Scallops * season	Between Groups	(Combined)	0.036	2	0.018	0.932	0.41
	Within Groups		0.389	20	0.019		
	Total		0.425	22			
Gastropod * season	Between Groups	(Combined)	150.009	2	75.004	1.353	0.281
	Within Groups		1108.929	20	55.446		
	Total		1258.937	22			
Green mussels * season	Between Groups	(Combined)	6911.609	2	3455.804	2.106	0.148
	Within Groups		32820.778	20	1641.039		
	Total		39732.386	22			
Modiolus * season	Between Groups	(Combined)	59763.716	2	29881.858	2.479	0.109
	Within Groups		241063.762	20	12053.188		
	Total		300827.478	22			
Nudibranchs * season	Between Groups	(Combined)	9.864	2	4.932	2.051	0.155
	Within Groups		48.097	20	2.405		
	Total		57.961	22			
Oyster * season	Between Groups	(Combined)	274.109	2	137.055	0.983	0.392
	Within Groups		2788.692	20	139.435		
	Total		3062.802	22			
Limpets * season	Between Groups	(Combined)	0.906	2	0.453	0.932	0.41
	Within Groups		9.722	20	0.486		

	Total		10.628	22			
Echinoids * season	Between Groups	(Combined)	0.431	2	0.216	0.042	0.959
	Within Groups		101.984	20	5.099		
	Total		102.415	22			
Ophiuroids * season	Between Groups	(Combined)	7.764	2	3.882	0.815	0.457
	Within Groups		95.27	20	4.763		
	Total		103.034	22			
Ascidians * season	Between Groups	(Combined)	3.828	2	1.914	2.37	0.119
	Within Groups		16.153	20	0.808		
	Total		19.981	22			

The ANOVA results obtained from SPSS (table.1), for the site and biofouling communities have shown no significance, but the season and the biofouling communities(table.2) have shown significance (P<0.05).

5.2 Short term seasonal succession studies (cage culture net)

In the cage net, hydroids, algae and *Modiolus* were the important foulers observed in the seasonal succession series. In the pre-monsoon season algae appeared in the panel followed by hydroids and *Modiolus* in less density along with other fouling organisms like sea anemones, barnacles, crabs, shrimps, Isopods, gastropods, green mussels, nudibranchs, oysters, echinoids, Ascidians. In the monsoon hydroids dominated the settlement, with reduction in algal settlement. *Modiolus* also appeared on the panel during monsoon. But the important foulers like sea anemones, shrimps, gastropods, nudibranchs and ascidians disappeared during monsoon, where as sponges and limpets appeared during the sesaon. In post monsoon, algae dominated and the *Modiolus* density also was increased. Whereas flat worms and ascidians disappeared. On the short-term panels in the premonsoon, ascidians appearedon the panel which were absent in the monsoon and post monsoon. Flatworms were also absent in the monsoon. It is observed that in the short-term studies on the culture nets, hydroids settlement was during the month of September. *Modiolus* settlement was in May, October, November, December and February month and algal settlement was during March and April.

5.3 Climax community

In literature of ecological succession, Clements theory is an idealistic theory proposing climax as the final stage of succession process.[33] In most of the Indian studies, climax species was *Perna viridis*, and in few studies barnacles and ascidians were climax communities[12]. Scanty literature is available on the Climax communities concerned to the aquaculture net panels. Sahu et al.[11] has reported Green mussels as the climax community on wooden panels. In the present study which was carried out in a culture farm, the green mussels formed the climax community. But in the reference site which was away from the culture activities no climax as such was observed. This may be due to the grazing and predation effect of wild fishes and other animals in the reference site. Green mussels were the dominant foulers forming the climax, due to their higher efficiency to hold the net fibers, fast growth and higher ability to filter the photo-planktons which are available in large quantities due to the higher nutrients' availability. These dominant biofoulers were successful in competing with other biofouling communities due to their larger size, growth, longer life span, longer larval stage.[11,34] In most of the aqua culture related biofouling studies carried out in Mediterranean, where sea bass is culture fish, the mussels, hydroids, algae were the dominating communities.[35] and the present study also endorse those results.

Fig 13. Green mussels forming the climax on the panel in sea cage farm

6. CONCLUSION

Hydroids were the initial and frequently occurring community, and green mussels formed the climax community on the long term panels of cage site. In cage net, highest settlement was in May dominated by hydroids and algae. Frequent net exchange was need to be carried out during summer months and immediately after spat settlement of green mussels (September-November), *Modiolus* (February). During initial months the grazing organisms and fishes can be introduced in the cages along with the culture fishes, so as to reduce the fouling by biological method. It is also suggested to use eco friendly organic artificial agents to overcome these fouling. Environmental friendly net service stations are available for cleaning and treating the fouled nets in many countries and it is suggested that similar facilities need to be developed in India also, to promote open sea mariculture.

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9. AUTHORS CONTRIBUTION STATEMENT

Mrs.Sonali S.Mhadolkar, conducted the research work and contributed to writing of the manuscript. Co Authors contributed to the analysis of the results and necessary inputs are given for the designing of manuscript.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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Impact of water quality parameters on diversity and intensity of biofouling at sea cage farm, Karwar, Karnataka, India

Mhaddolkar Sonali S[1], Dineshbabu AP[2], Thomas Sujitha[2] and Loka Jayasree[1]

[1]Karwar Research Centre of Central Marine Fisheries Research Institute , P.O. Karwar, Uttarkannada, Karnataka, India 581 301
[2]Mangalore Research Centre of Central Marine Fisheries Research Institute, PB No.244,Hoige Bazar, Mangalore
Email: sonamdkar@gmail.com

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ABSTRACT

Marine cage farming is becoming a viable method for marine food production and the economical viability of the production is very much influenced by infestation of biofoulers. The distribution and abundance of biofoulers depends on the environmental and hydrographical parameters of the water body. Present study was carried out to understand the impact of different parameters on the composition of the biofouling communities at sea cage farm at Karwar, India. Physico-chemical parameters were studied by taking samples from reference & cage site, at 1m, 3m and 6m depths. Even though there was no significant variation in the water quality parameters of two locations, community compositions of the biofoulers are reported to have significant correlation with the various environmental parameters. For example, Nitrite has showed a significant positive correlation with Hydroids and Sea-anemones. Many of the biofoulers showed significant positive correlation with other foulers. Present study was also attempted to understand the interdependency of the fouling species along with their dependency on the environmental parameters. Information on the correlation of environmental parameters and biofouling characteristics will help to ensure feasibility of the fish farming operations by taking appropriate decisions on site selection, duration and period of cage farming etc.

Key words: biofoulers, correlation, community composition, environmental parameters.

INTRODUCTION

Study of hydrological parameters has gained importance because these hydrological parameters affect greatly on the abundance, occurrence and survival of the biofouling organisms. As favorable water quality parameters

are prerequisite for plankton production which indirectly effects on the growth of biofoulers in the sea. The literature study on the Karwar hydrological parameters gives an insight on contribution of researchers (Ramamurthy, 1963; Noble, 1968; Annigeri, 1968, 1972; Radhakrishnan *et al*., 1978; Philipose *et al*., 2014) on hydrology study.

Salinity, Temperature, pH, Dissolved oxygen and Ammonia are considered as very important parameters in aquaculture practices (Pitta *et al*., 1999; Wu *et al*., 1999; Karakassis *et al*., 2001). Fouling rate on submerged marine substratum varies with the environmental parameters such as Temperature, Salinity, nutrients and current velocity etc. The influence of physico-chemical parameters on *Tubularia larynx* (hydroid) was studied by Nellis (1996). The community compositions of the biofoulers are reported to have significant correlation with the various environmental parameters (Fitridge *et al*., 2012).

Water quality studies of Indian cage culture sites were carried out by many authors (Imelda *et al*., 2010; Anil *et al*., 2010; Prema *et al*., 2010; Philippose *et al*., 2010, 2012). Earlier studies on variations of environmental parameters of sea cage farm of Karwar in west coast were conducted by Philipose *et al*.(2010). But the seasonal correlation studies between the Cage environmental parameters and biofouling is the first of its kind in Indian mariculture studies. Here more emphasis is given on the correlation between the cage environmental parameters and the biofouling community structure. So this study is very important in cage culture research in Indian waters. The main objective to study the hydro biological parameters of the study sites is to understand the trend of different parameters which are influencing on the composition of the biofouling communities. Along with the biological factors like grazing, competition for space and various other factors ,these hydrological parameters are controlling the biofouling intensity.

MATERIALS AND METHODS

This study was conducted at the fish cage farm, located at N 14°48.406' & E 074°06.664' in Karwar, Karnataka, west-coast of India, in the Arabian Sea (Fig.1.a &b). Area of marine cage farm is about 1.5 Km^2 and depth 8-10m. It holds floating circular cages ranging from 3m - 12m diameter size. Finfishes like Mangrove redsnapper- *Lutjanus argentimaculatus [Forsskal],* Johns snapper- *L. johnii [Bloch],* Pompano- *Trachinotus blochii [Lacepede],* Cobia-*Rachycentron canadum [Linnaeus]* and Seabass- *Lates calcarifer [Bloch]* were being cultured so far.

Physico-chemical parameters like Temperature, Salinity, Dissolved oxygen, pH, Ammonia, Nitrite, Phosphate (Gouri *et al*., 2011) were estimated by standard methods (APHA, 2007). Temperature was measured by mercury thermometer ($\pm 0.01^0$C accuracy) and Salinity is measured by Knudsen's method. Dissolved Oxygen is estimated by Winkler's Method and pH, is measured by using the pH probe (Multi parameter-Test-35-series). The nutrients Ammonia, Nitrite, and Phosphate were estimated by using colorimetric (Strickland and Parsons, 1975) and Spectrophotometeric method. Monthly and Seasonal variation in Salinity, Temperature, Dissolved oxygen, pH, Ammonia, Nitrite and Phosphate were studied in all the three depth where the experimental panels were deployed at cage and reference site.

Biofouling density and diversity was analyzed by taking monthly and seasonal data from the cumulative experimental panels of cage and reference site for the year 2015-2016. Rectangular experimental panel set-ups with total 72 HDPE net pieces of $10cm^2$ (mesh =22mm) size were fixed to three PVC frames, deployed at 3 depths viz. 1mt,3mt,6mt(top, middle, bottom) each in cage and reference site. The panels were submerged vertically in the water column by using float and anchors. Reference selected was 500 meters away from cage farm.

The data collected was analyzed statistically using software like SPSS, PAST, compared using two way ANOVA. The correlation with the seasonal variation of hydrography, fouling diversity and fluctuations in the growth of fouling organisms were studied. The biofouling community structure and seasonal variations were analysed using the PAST software. The biofoulers seasonal correlation between the water quality parameters and the diversity indices were analyzed with CCA (canonical-correspondence-analysis). The detailed study about the correlation between each community and the water quality parameter is further studied using the Two tailed Pearson correlation analysis, using SPSS software.

Fig.1. A : Cage farm of Karwar where the panel experiment was conducted**, B:** Experimental panels designed for biofouling studies

RESULTS AND DISCUSSION

The water quality parameters like variations in Salinity, Temperature, Dissolved oxygen, pH, Ammonia, Nitrite and Phosphate were collected to understand the relationship between these parameters and fouling. The correlation with the seasonal variation of hydrography, fouling number and diversity will be useful in understanding the influence of environmental parameters on the large scale fluctuations in the growth of fouling organisms.

Water quality parameters:

Seasonal variations in water quality parameters are analyzed between the two sites (fig.2). ANOVA study has shown that there is no significant variation in the water quality parameters and depth of two sites. Water quality studies of Indian cage culture sites were carried out by Imelda *et al*., (2010); Anil *et al*., (2010) and Prema *et al*., (2010) including studies of the same sea cage farm (Philipose *et al*.2012).

Seasonal variations in Temperature ranges have shown similar trend in cage and reference sites i.e. Temperature was low in monsoon near bottom panel and higher at top panel during pre monsoon. One way ANOVA results showed there is no significant difference between the sites but there is significance between monsoon and pre monsoon seasons ($p \leq 0.05$). The general studies on Monthly variations of water quality parameters of Karwar waters (Noble, 1968) observed higher Temperature ranges at deeper water, during the month of March and May. But overall the Temperature values were found slightly lower towards the depth at Karwar waters (Annigeri, 1966). In present study the deeper water during March and May month has showed higher ranges of Temperature, supporting the earlier observations of Noble (1968). and the studies by Philipose *et al*. (2012) in the cage farm covered under present study. In the present observations the highest Temperature was found to fluctuate from May to March. But in reference site similar observations were made as the previous studies of the same sites and the peak Temperature was observed during May. At the cage site two peaks have been observed during March and May 2015 with significant variation in Temperature among the sites. Comparatively higher Temperature ranges were observed in the cage site compared to the reference site this may be due to the influence of the rocky stretch of break water near the cage site.

Seasonal Salinity was low at top panel during monsoon high near bottom panel during post monsoon at both the sites. Comparatively Salinity is less in the cage site, this may be due to the effect of the estuarine fresh water influx from Kali estuary. One way ANOVA results showed there is significance between seasons ($p \leq 0.05$). The earlier works state that significant variations have not observed between the stations. Our present results also support the same. Seasonal Salinity ranged from 19.97 ‰ near top panel during

monsoon to 33.11‰ near bottom panel during post-monsoon in the cage site. In the reference site the Salinity varied from 25.28 ‰ during monsoon near top panel to 32.86 ‰ in post-monsoon near bottom panel.

Dissolved oxygen in water was maximum during post monsoon near top panel of both cage and reference site. DO was low during monsoon near bottom panel at the cage site, but in the reference site it is low during pre-monsoon near bottom panel. DO levels were lower in the cage site when compared to the reference site. Trends in Dissolved oxygen accord with the results of previous workers (Annigeri, 1966). During July the values were the lowest & May it was highest at the cage site (Philipose *et al*., 2012). In the reference site the peak values were recorded in the month of August 2016 in the toper layers of water column. Significant difference was not found between the stations in the earlier studies. The lowest value in monsoon in all the panels of cage site, may be due to the increased zooplankton density in water. The higher levels of DO in reference may be due to the current pattern and wave action.

In both the sites pH was low near bottom panel during monsoon and high during post monsoon near bottom panel at the cage site, and top panel of the reference. pH levels were higher in the reference site compared to the cage site, in top and middle panels but in bottom panels it is reverse in all the seasons. pH values were in the similar range (Philipose *et al*.2012) as that of earlier workers except during January and September in cage and reference site respectively.

Ammonia was maximum during monsoon near bottom panel in both the sites, the highest was 0.28 mg/l, in bottom waters near the cage panels. This may be due to low Salinity, the disintegration of most of the marine fouling species has taken place in monsoon. The minimum Ammonia values were observed in pre-monsoon season near middle panel in the reference site and top, middle panels at the cage site. The present values of ammonia were higher compared to the earlier works of 2009-10 (Philipose *et al*., 2012) in the same site.

Overall the higher ranges of nitrite were observed in cage site. Nitrite ranges were low during pre-monsoon season near bottom panel and maximum near middle panel at the cage site, In the reference site the nitrite was low during Monsoon near middle panel, higher in post monsoon near bottom panel. In the monsoon season highest nitrite values were observed in cage site. During 2009 and 2010 (Philipose *et al*., 2013) values ranged between 0.1 -0.6mg^{-1}, but in present period the values ranged between 0.00 to 1.09mg^{-1}. In reference site during September depletion of nitrite was observed in the three depths and the values of nitrite were comparatively higher in the cage site when compared with the reference site. This may be due to the higher nutrients in the cage due to artificial and live fish feed given to the cultured fishes in the cages. The reference site is free of added nutrients as feed, the values of nitrite are lower in the present case.

Phosphate ranges are higher during pre monsoon near top panels at the cage and reference sites. Phosphate ranges were low near middle panel, during post monsoon in cage site and pre-monsoon in reference site. Phosphate values were high in the cage site in all the seasons when compared to the reference site. Ammonia, nitrite and phosphate levels were higher in the cagesite when compard to the referance site.

Correlation between the water quality parameters and the fouling organisms:

The results of two tailed Pearson correlation analyzed in SPSS software were presented in table 1. The cnidarians like hydroids and sea anemones have showed significant negative correlation ($p \leq 0.01$) with pH and positive correlation with Nitrite. Ascidians showed a significant negative correlation with Salinity, Dissolved oxygen ($p<0.01$) and Nitrite ($p<0.05$). Echinoderms showed significant negative correlation with Nitrite ($p<0.05$). *Gouri et al*. (2015) analyzed that Salinity effects the biofoulers settlement in the Kalpakkam waters. Swami and Udaykumar (2008) observed effect of pollution on reducing the biodiversity in two different localities, and suggested influence of Salinity and pollution on structure and composition of boifoulers. Also there are reports of faster development of foulers (FAO, 1989) with higher Salinity, Temperature and nutrients. The blockages of meshes of cage net due to fouling leads to the low Dissolved oxygen level inside the cage and the influence of these parameters on hydroid *was* studied by Nellis (1996).

Environmental parameters like Temperature and Salinity have impact on development and recruitment of biofoulers (Anil *et al*., 1996). Nasar (2017) while

investigating biofoulers assemblage on experimental panel in two locations observed nutrients play important role in development of communities and described the biofouling pattern by combined effect of water quality parameters (Temperature, Ammonia, Nitrite and Phosphate). The flatworms and barnacles showed positive correlation (r=0.841, at 0.01 significance level) the similar observations were made in case of Gouri *et al*.(2015) in the Kalpakkam waters ,on the teak wood panels in bay of Bengal.

Assemblage of fouling organisms varies in different depth of the water column. It is due to the variation in light penetration, Salinity, Temperature, DO, pH, water current and physico chemical characteristics. The physical and biological characteristic along the water column brings out some systematic responses among the fouling organisms. A special temporal variations result into the deferral assemblage composition which differs over time and space.

Biofouling is mainly contributed by Cnidarians (hydroids) and hydroid showed a significant negative correlation ($p< 0.01$) with pH and showed a significant positive correlation with Nitrite (0.598). Ascidians showed a significant negative correlation with Salinity i.e. the number of ascidians decreased with the increase in Salinity.

The hydroids, sea anemones, echinoids and ascidians showed significant negative correlation with pH i.e. when the pH is low the theses communities were more in number. Nitrite level influences the number of Hydroids and sea anemone i.e. they have showed significant positive correlation with nitrite. It is also concluded from the results that when the Nitrite level is high and pH and DO are less hydroids were abundant.

Salinity, DO, pH exhibited significant negative correlation with Ascidians, Hydroids, Sea anemones, Echinoids, So as to influencing the biofouling community structure. Similarly Nitrite has showed a significant positive correlation with Hydroids and sea anemones, influencing the biofouling community structure in the Karwar waters. The similar results have been obtained by Pati and Balaji (2015) where they concluded that BOD, Salinity, Nitrite, Silicate and Phosphate were influencing water quality parameters determining the community structure of biofoulers in Vishakhapatnam Harbor.

Most of the biofoulers are having significant positive correlation with other foulers. Sponges showed significant positive correlation with Hydroids and echinoderms it was seen that sponges have grown on the hydroids and whenever the hydroids population is more on the panels echinoderms were found grazing on them. Sea anemones showed significant positive correlation with echinoderms, like hydroids sea anemones were also grazed by echinoderms (they attracted the echinoderms). Bryozoans have significant positive correlation with Isopods, because the isopods have been seen near the bryozoans in clusters. Flat worms have showed significant positive correlation with barnacles, It is seen that the flat worms were more abundant near the barnacle shells and ascidians may be they are taking the food particles or getting shelter. On the other hand Barnacles have significant positive correlation with Ascidians. Ascidians were found near the barnacle shell, there may be some kind of association with these two (association unknown). Shrimps and modiolus have significant positive correlation may be shrimps are feeding on the modiolus. So their number is showing positively significant correlations. Scallops have significant positive correlation with Nudibrachs. Nudibranch will feed on the bivalves like scallops. Oysters have significant positive correlation with Limpets. Limpets and oysters are seen more on the surfaces of barnacles and both will prefer same type of substratum and similar kind of water quality parameters and depth.

The ANOVA results obtained (SPSS) for both site (cage and reference) and biofouling communities has showed no significance, but between the season and the biofouling communities has shown significance ($P<0.05$). Dehmordi *et al*. (2011) worked on the parameters influencing the diversity the biofoulers of port of Deilam, Iran, found that it varied with Temperature and other parameters like Salinity, pH, conductivity had no significance on diversity. The Bryozoans and the isopods were showed significance between the seasons, influencing the structure of the biofouling community. The community compositions of the biofoulers are reported to have significant correlation with the various environmental parameters (Fitridge *et al*. 2012). Pati *et al.* (2015) concluded that biofouling community structure is mainly influenced by BOD, Salinity, Nitrite and Phosphate. And the similar results were obtained with the present study.

Table 1. Correlation between hydrological parameters and Biofoulers:

	Temp	salinity	DO	pH	Ammonia	Nitrite	Phosphate	Sponge	Hydroid	eaanemon	Bryz	Flworm	Poly	mphipod	Bar	Crab	Shrimps	Isopod	Scallops	astropod	Gmussl	Modiolus	Nudi	Oyster	Limpets	Echinoids	phiuroids	Ascidians
Temp	1	0.335	-0.058	0.308	-0.374	.517*	0.238	-0.178	-0.077	-0.033	0.095	-0.298	0.195	0.323	-0.348	-0.145	-0.12	0.183	-0.141	0.24	-0.081	-0.12	0.024	-0.329	-0.332	-0.086	0.008	-0.205
Salinity		1	.599**	.655**	-0.291	-0.083	0.008	-0.183	-0.195	-0.101	0.006	-0.122	-0.054	0.29	-0.166	-0.175	0.048	0.123	0.076	-0.241	0.266	0.096	0.103	-0.207	-0.192	-0.232	0.008	-.651**
DO			1	.758**	-0.31	-0.306	-0.234	0.201	-0.194	-0.15	0.085	-0.368	-0.102	0.098	-0.377	-0.127	0.109	0.339	0.084	-0.285	0.114	0.154	0.068	0.138	0.135	-0.312	0.068	-.630**
pH				1	-.430*	-0.319	-0.184	-0.041	-.544**	-.513*	-0.024	-0.244	-0.072	0.21	-0.323	-0.218	-0.156	0.291	0.109	-0.112	0.152	-0.036	0.125	0.021	0.062	-.453*	-0.132	-.433*
Ammonia					1	-0.314	.500*	0.043	0.111	-0.231	-0.083	0.176	-0.126	-0.336	0.304	0.265	0.224	-0.356	-0.169	-0.2	-0.006	0.176	-0.224	0.068	0.086	-0.031	0.152	0.079
Nitrite						1	-0.024	0.209	.598**	.686**	0.303	-0.181	0.065	0.08	-0.217	-0.059	-0.053	-0.025	-0.119	-0.041	-0.066	-0.193	0.197	-0.216	-0.267	0.325	0.002	0.022
Phosphate							1	-0.131	-0.11	-0.212	0.051	0.232	-0.201	-0.067	0.307	0.108	0.364	-0.093	-0.132	-0.094	-0.005	0.338	-0.191	-0.158	-0.14	-0.113	0.348	0.186
Sponge								1	.556**	0.319	0.335	-0.03	-0.096	-0.181	-0.102	-0.165	-0.097	0.037	0.098	-0.158	-0.112	-0.132	0.258	-0.061	-0.033	0.222	-0.115	-0.056
Hydroid									1	.752**	0.393	0.029	0.019	-0.104	0.042	-0.09	-0.118	0.048	-0.108	-0.257	-0.226	-0.241	0.077	-0.144	-0.177	.441*	-0.131	-0.031
Seaanem										1	0.171	-0.101	0.102	0.042	-0.075	0.036	-0.005	-0.072	-0.081	-0.125	-0.105	-0.111	0.071	0.014	-0.081	.597**	0.008	-0.109
Bryz											1	0.193	0.334	-0.063	0.068	0.288	-0.151	.601**	0.085	-0.152	-0.097	-0.196	0.395	-0.152	-0.139	-0.142	-0.16	0.14
Flworm												1	0.147	0.097	.841**	-0.019	-0.077	0.085	0.412	-0.112	-0.069	-0.066	0.343	-0.103	-0.065	-0.169	-0.088	.584**
Poly													1	0.406	0.024	0.302	0.005	0.344	0.094	0.409	-0.196	-0.029	0.183	-0.074	-0.085	-0.012	0.092	0.043
Amphipod														1	0.153	0.087	-0.116	-0.028	-0.095	0.147	-0.248	-0.168	-0.065	-0.046	-0.033	0.222	-0.087	0.017
Bar															1	0.131	-0.043	-0.192	-0.091	-0.113	-0.076	-0.02	-0.123	0.066	0.079	-0.165	-0.065	.624**
Crab																1	0.18	-0.059	-0.253	-0.118	-0.044	0.189	-0.041	0.167	0.088	0.014	0.267	0.102
Shrimps																	1	-0.144	-0.054	0.002	0.375	.938**	-0.076	-0.073	-0.054	-0.077	.941**	-0.098
Isopod																		1	0.401	-0.108	-0.139	-0.079	0.374	-0.124	-0.128	-0.263	-0.076	-0.1
Scallops																			1	-0.079	-0.098	-0.069	.831**	-0.072	-0.045	-0.117	-0.061	-0.083
Gastropod																				1	-0.098	-0.016	-0.112	-0.122	-0.079	0.012	-0.014	0.22
Gmussl																					1	0.395	-0.023	-0.116	-0.081	-0.221	0.34	-0.094
Modiolus																						1	-0.098	-0.103	-0.069	-0.129	.888**	-0.098
Nudi																							1	-0.101	-0.064	-0.166	-0.087	-0.075
Oyster																								1	.975**	-0.106	-0.039	-0.122
Limpets																									1	-0.117	-0.061	-0.083
Echinoids																										1	-0.115	0.057
Ophiuroids																											1	-0.112
Ascidians																												1

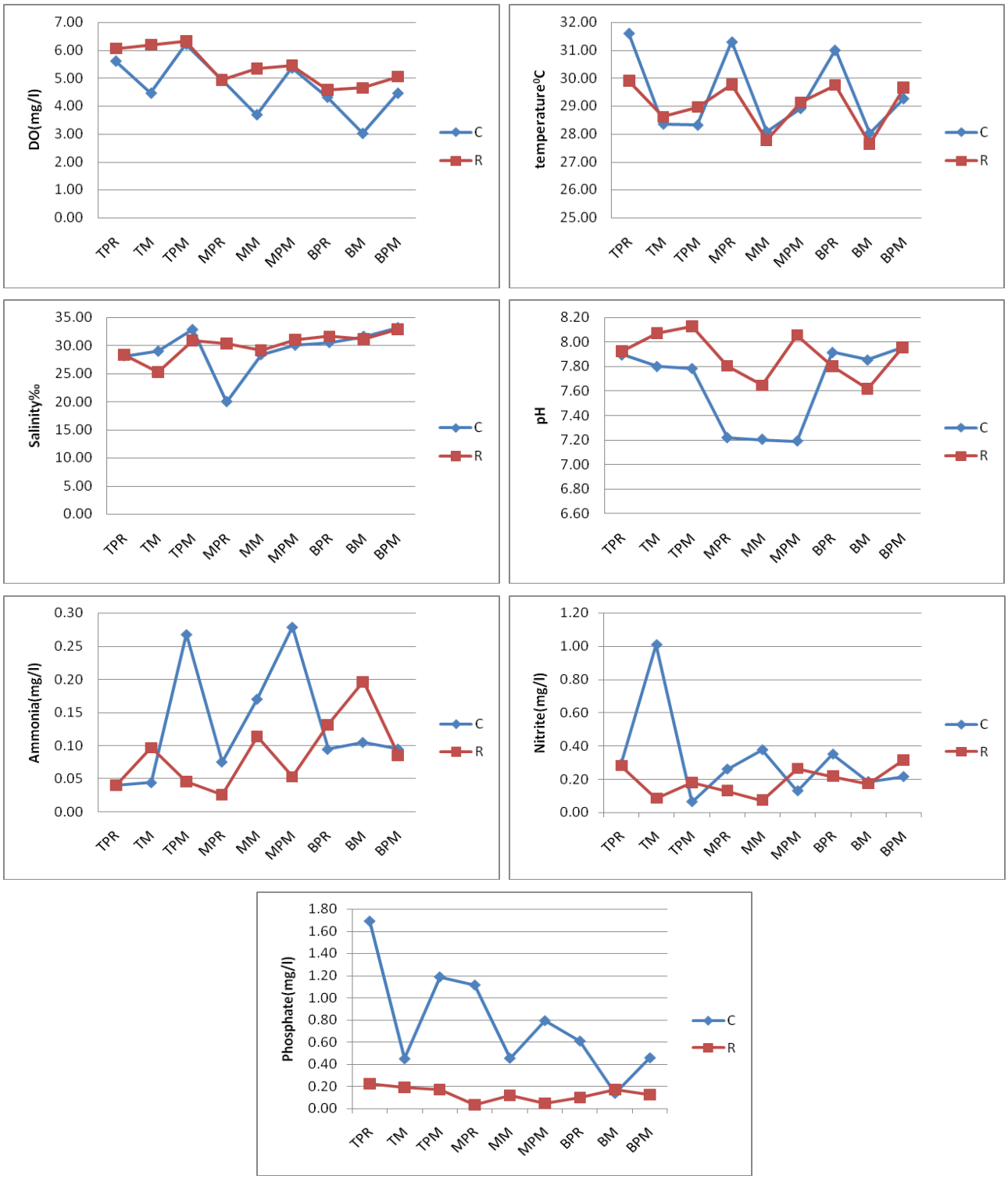

Fig.2. Seasonal variation of hydrological parameters at cage and reference experimental panel [TPR-top premonson, TM-top monsoon, TPM-top post monsoon, MPR-middle premonsoon, MM-Middle monsoon, MPM-middle post monsoon, BPR-Bottom premonsoon, BM-bottom monsoon, BPM-Bottom post monsoon].

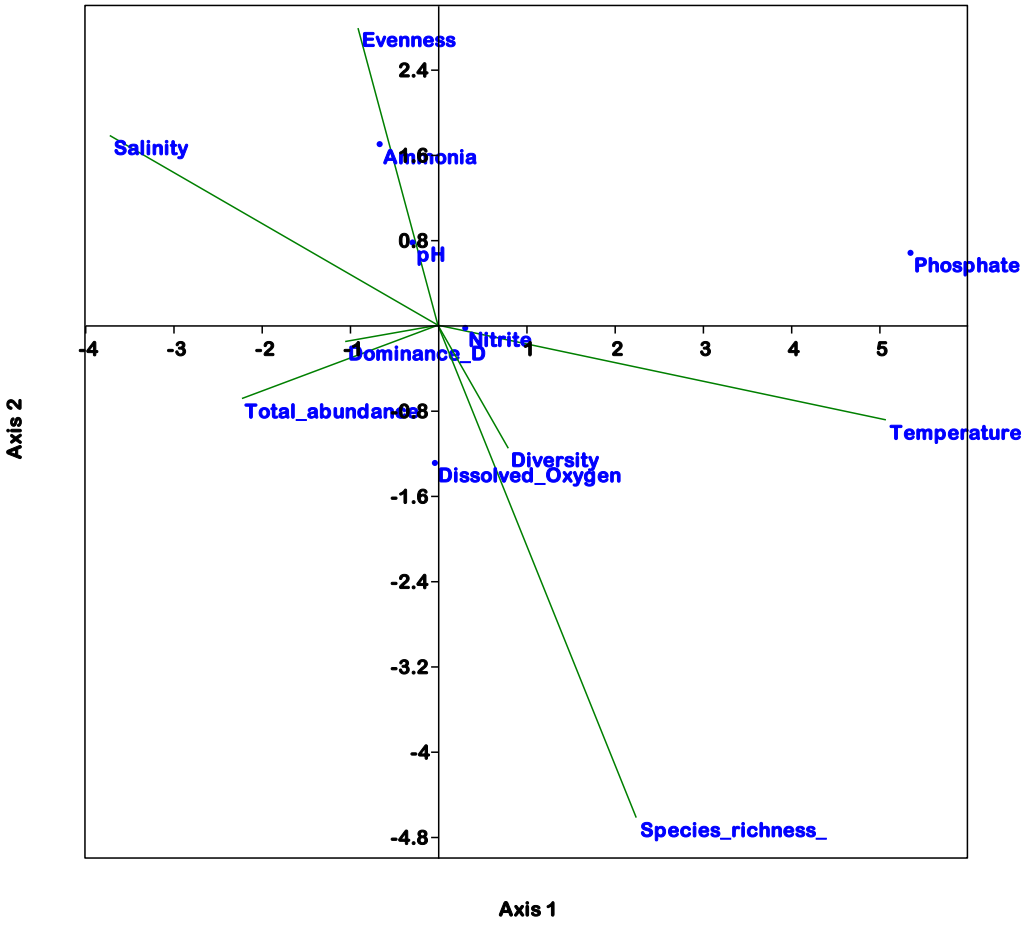

Fig. 3. CCA showing relation between water quality and biofouling community indices.

Relationship between environmental parameters and Diversity indices of fouling communities

Seasonal biodiversity Indices of both the sites were studied by taking the density of biofoulers. Low abundance (97no10cm^{-2}) in middle panels in cage during monsoon and high (1685no10cm^{-2}) in reference pre-monsoon panel. Diversity in Experimental panels of cage site during premonsoon was low in Bottom (BPR) and higher in Middle (MPR) panel. Dominance in cage site during pre-monsoon was low in middle (MPR) panel and high in the bottom (BPR) panel. Evenness was low in bottom reference pre-monsoon (BPR) and high in bottom cage monsoon (BM) panel. Species richness was low in Bottom cage post-monsoon, (BPM) and high in Middle cage pre-monsoon (MPR) panel.

The CCA Graph is generated from PAST using canonical correspondence showing relationship between environmental parameters and seasonal abundance of biofoulers in different sites and depths. The results obtained using CCA of relationship between the water quality parameters (Salinity, Temperature, Dissolved oxygen, pH, Ammonia, Nitrite, Phosphate) and biofouling community indices (dominance, diversity, evenness and total abundance) are presented in Fig.3.

When the Temperature, phosphate levels were high the species richness was relatively high, when the Salinity and the ammonia levels was more, the evenness was relatively more. The species richness is low when the Salinity is lower. Diversity was more when DO, nitrite, levels were relatively high. Fouling rate varies with the environmental parameters such as Temperature, Salinity, nutrients and current velocity

etc. Salinity, Temperature, pH, Dissolved oxygen and Ammonia are considered as very important parameters in aquaculture practices (Pitta *et al*., 1999; Wu *et al*., 1999; Karakassis *et al*., 2001).

According to well known principal of Maguran (1988), biofoulers diversity and evenness decreased with intensification of dominance. In present investigations the dominance of biofoulers was contrary to the evenness and diversity. Similar results were obtained in wooden panel studies of biofoulers in Vishakhapatnam Jetty (Pati *et al*., 2015).

CONCLUSION

When DO, Temperature is high the diversity is more and when the Salinity and Ammonia levels are high evenness is more. The water quality parameters influence the diversity, fouling community structure and composition of biofoulers. Seasonal studies showed that Ammonia levels were low during pre-monsoon and high during monsoon. Highest diversity and density was recorded in pre-monsoon. Because of the Salinity changes, the disintegration of most of the fouling species, high ranges of Ammonia were towards the bottom during the monsoon in both the sites. In the cage site because of the external feed and fecal matters of culture fishes the Ammonia levels are higher than the reference site.

Biofouling is mainly contributed by cnidarians (hydroids) and showed a significant negative correlation with pH and significant positive correlation with nitrite. Ascidians showed a significant negative correlation with Salinity i.e. the number of ascidians decreased with the increase in Salinity and also most of the biofouling communities are having significant positive correlation other foulers. The analysis of relationship between the water quality parameters and biofouling diversity studies revealed that Temperature, Salinity, DO, pH and Nitrite can be considered as major water quality parameters influencing the community structure and diversity of biofoulers in Karwar cage farm.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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Sonali S Mhaddolkar
Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar, Uttara kannada, Karnataka, India

Jayasree Loka
Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar, Uttara kannada, Karnataka, India

KK Philipose
Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar, Uttara kannada, Karnataka, India

AP Dineshbabu
Mangalore Research Centre of Central Marine Fisheries Research Institute, Hoige Bazar, Mangalore, Karnataka, India

Correspondence
Sonali S Mhaddolkar
Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar, Uttara kannada, Karnataka, India

Experimental studies on Macro fouling communities on net panels at marine cage farm of Karwar, India

Sonali S Mhaddolkar, Jayasree Loka, KK Philipose and AP Dineshbabu

Abstract
A study was undertaken on the fouling pattern, monthly settlement and species dominance at marine cage culture during the year 2014-2015. The net panels deployed at three different depths during December 2014 and the study was carried out for seven months. The study revealed wide variations in colonization of the bio-foulers, the density varied between 2-260/cm^2. The overall density was found to be more in bottom panels (6mt) compared to the top (1mt) and middle panels (3mt). The overall dominant species were hydroids and barnacles throughout the culture period. In the top panels, hydroids were dominant (91.67%) during December, followed by barnacles and amphipods. Additionally, complete dominance of barnacles was observed in the middle panel during May. Barnacles were found dominant (93.75%) on the bottom panels during March, followed by hydroids and crabs. The study underscored that barnacles formed a major contribution of the fouling organisms throughout the culture period. Significant variation in the occurrence and abundance of fouling organisms was observed, between the depths at cage site.

Keywords: Bio-foulers, cage farm, density, dominance

1. Introduction
Cage Bio-foulers are the organisms which are responsible for the degradation of cage accessories such as cage frame, nets, barrels, ballast and ropes. The growth of fouling organisms severely affects the net of cages and so the culture activities. Worldwide many authors [1, 2] made significant contributions on species composition of fouling organisms on mariculture cages. In India, cage culture of marine finfish and shellfish has been initiated in 2007 by Central Marine Fisheries Research Institute and successfully cultured Asian sea bass. CMFRI has developed a Marine farm at, off Karwar bay for culturing Finishes and shell fishes. During the study period there were 25 cages in the marine farm culturing Cobia (*Rachycentron canadum*), Pompano (*Trachinotus blochii*), Seabass (*Lates calcarifer*) and red snapper (*Lutjanus argentimaculatus*). Very little work has been carried out in India on biofouling communities on marine cages [3]. Therefore, the present study has undertaken at marine farm of Karwar, to assess the fouling pattern, monthly settlement and species dominance in cage culture site of west coast of India during the period of 2014-2015.

2. Materials and Methods
The marine farm of CMFRI is located at N 14º48.406', E 074º06.664' in the west coast of India (fig.1). The net panels (100mmx100mm) of mesh size 22mm were deployed in cage at 3 different depths during December 2014 and the field and laboratory studies were carried out for a period of seven months during the culture period. The panels were submerged for one month and replaced with new panels after every sampling. Duplicate panels were put at three depths (1m, 3m and 6m) based on transects made vertically for cage depth. The panels were tied to the floating frames of the cage farm at the selected points. Monthly data was collected and the species were counted by number of individual organisms per cm^2. The fouling organisms on the panels were brought to the laboratory in sea water, washed with sterile sea water and the fouling organisms are sieved in 200 micron sieve, and the fouling samples were preserved in Rose Bengal and 5% formaldehyde solution for further identification. Smaller organisms were observed under 5x magnifications in AXIO, Zeiss (Scope-A1) microscope. Identifications up to possible lowest taxonomical rank were done by using World register of marine species [4].

Fig 1: Marine cage farm at Karwar, west coast of India

3. Results and discussion:

Totally 7 phyla were found on the cage panel nets, viz. Porifera, Coelenterata, Mollusca, Annelida, Arthropoda, Echinodermata, Chordata. A total of 21 macrofouling organisms were identified from the marine cage farm during the study period (Table 1).This number is similar to the earlier literature of fouling communities on net panel in Norwegian fish farm [5] where as 28 species were identified during seven-months period at 3 different depths.

Table 1: Macro-fouling organisms identified from the marine cage farm

Macrofouling organisms	Species identified	Phylum
Bryozoans	*Bugula neritina*	Bryozoa
Hydroids	*Obelia* sp.	Coelentrata
Sea anemone	*Bunodosoma* sp.	Cnidaria
Gastropod	*Oliva inspidula*	Mollusca
Green mussel	*Perna viridis*	Mollusca
Modiolus	*Modiolus* sp.	Mollusca
Oyster	*Crassostrea madrasensis, Saccostrea cucullata*	Mollusca
Polychaete worms	*Platynereis* sp.	Annelida
Flat worm	*Leptoplana* sp.	Annelida
Amphipod	*Hyperia* sp. *Caprella* sp.	Arthropoda
Barnacles	*Balanus* sp.	Arthropoda
Crabs	*Grapsus albolineatus, Schizophrys aspera, Ozius rugulosus, Medaeus granulosus, Plagusia squamosa*	Arthropoda
Isopods	*Idotea* sp.	Arthropoda
Sea urchin	*Stomopneustes variolaris*	Echinodermata
Ascidia	*Ascidia* sp.	Chordata

Among the fouling organisms recorded during the study period, amphipods, barnacles, crabs, gastropods, hydroids, modiolus, polychaete worms and sea urchin were found to be common from all the three depths. It was found that barnacles dominated in the middle and bottom panels whereas, hydroids dominated the top panels.

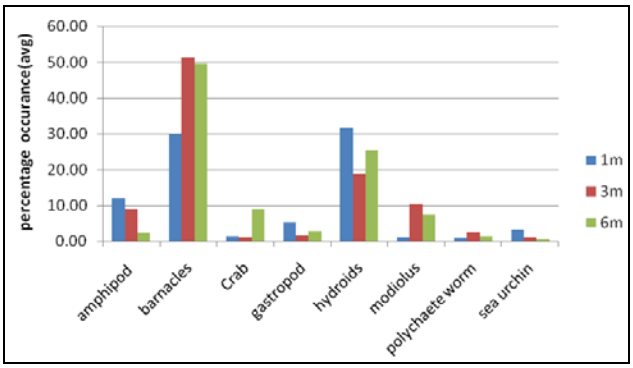

Fig 2: Showing the Macro fouling organisms recorded from the panels of 3 depths in marine farm.

Hydroids were abundant during in December and January months in all the three panels, earlier studies of fouling showed that there are serious problems of fouling of hydroid dominance on culture nets from the month of July to November in Norwegian waters [6, 7, 5]. Carl [6] reported that according to survey conducted by him, fouling colonisation showed a shift starting from top to bottom and bottom to top. In the present Investigation, most of the organisms recorded in the fouling communities are sediment dwelling and rock dwelling communities and this could be due to the sheltered rocky environment where the marine farm is situated. The similar results were obtained in the earlier works [5] in the Norwegian waters.

3.1 Top Penal (1mt)

On the top panel, a total of 15 macro-fouling organisms viz., amphipods, ascidians, barnacles, gastropods, green mussels, bryozoans, hydroids, crabs, isopods, modiolus, oysters, flatworms, polychaetes, sea anemone and sea urchins were recorded during the culture period. Month wise percentage of dominant species was recorded (Fig.3.). Overall, hydroids (31.56%) were found to be the dominant species on the top panels followed by barnacles 29.74% dominated during March, May, June, and July. Sea anemones were least dominant species on the top penal. The highest percentage was of hydroids (91.67%) in the month of December followed by barnacles (83%) in July and amphipods (70%) in February.

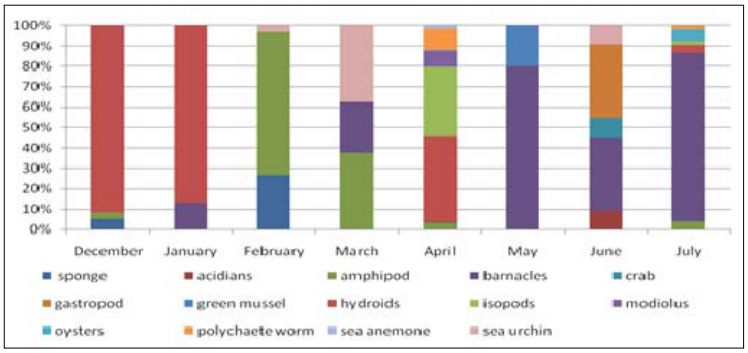

Fig 3: Showing the percentage of macrofouling organisms on the top penal.

3.2 Middle Panel (3mt)

In the middle panel, 13 macro fouling organisms namely amphipods, barnacles, crabs, sponge, gastropods, hydroids, flat worms, caprellids, green mussel, modiolus, polychaete worms, sea urchin and bryozoans were recorded. Monthly panel study showed barnacles as the most dominant group on the panel (50.48%) (Fig.4.). In the middle panel, Barnacles were present throughout the period of study with a complete dominance during the month of May.

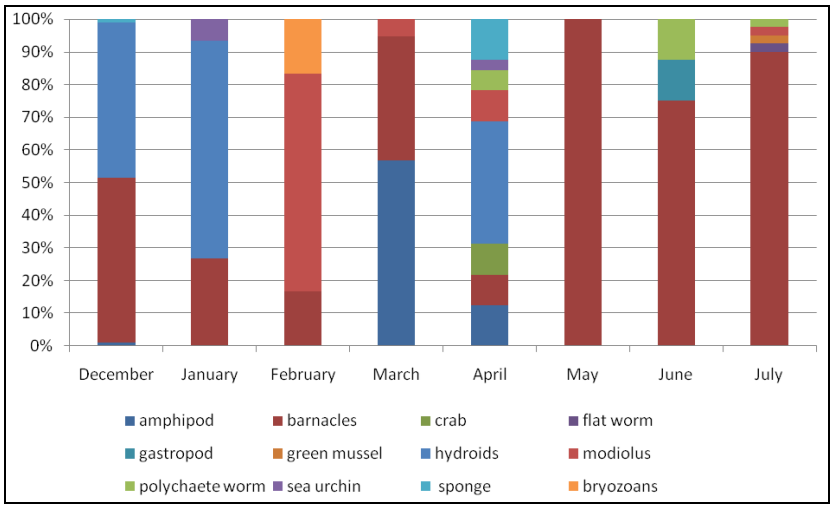

Fig 4: Percentage of Macrofouling organisms on the middle panel.

3.3. Bottom Panel (6mt)

In the bottom panel, a total of 9 macrofouling organisms were recorded viz., amphipods, barnacles, gastropods, crabs, hydroids, modiolus, polychaete worms, isopods and sea urchin. Month wise percentage of dominant species was recorded and (Fig.5.) found barnacles throughout the culture period except in the month of February with a peak of 93.75% during March. Lowest percentage was of sea urchin that is (3.13%) which was only found during April. Overall barnacles were abundant (49.63%) followed by hydroids (25.41%). The sea urchin was recorded as the least dominant with 0.39% occurrence.

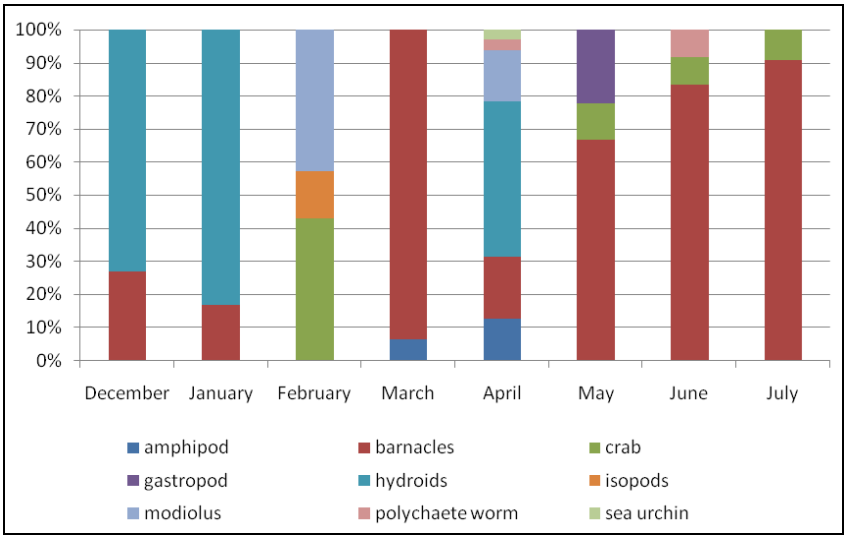

Fig 5: Showing the percentage of Macrofouling organisms on the bottom panel.

The present investigation showed that the overall percentage of fouling with barnacles and hydroids was 29.74%, 51.25%, 49.63% and 31.56%, 18.58%, 25.41% on top, middle and bottom panels respectively. The density of bio-foulers varied between 2-260/cm^2. It was reported that regular cleaning of nets can prevent the early stages of bio-fouling succession, mainly the first bio-fouling colonisers like Hydroid [7, 8, 9] in marine cage farms. It was found that there is a significant variation in the occurrence of species between three depths (p<0.05). Earlier reports also showed a significant variation in the assemblage of fouling organisms on the net panels with the depth [10, 11, 6]. This variation can be because of many factors such as temperature, salinity, light intensity, nutrient availability, dissolved oxygen conditions and current of the water [5]. Jennifer [12] studied succession development of fouling communities on open ocean aquaculture fish cages in the western Gulf of Maine, USA. It was observed that the hydroids were dominated in the early months of culture during the present investigation. Blocher, [14, 13] and Jemimah EK [5] also recorded that Hydroids dominated in early months which was latter replaced by barnacles.

Barnacles are reported to breed round the year [15, 16] and

among the macro-fouling communities barnacle are most dominating macro-foulers along the Indian coasts [18, 16]. Present studies revealed that overall; the barnacles are the most dominating fouling organisms on the net panel at 3mt and 6mt depths in the marine cage farm at Karwar. In east coast, Kaplakkam waters, panel studies have shown similar results [17].

4. Conclusions

The study revealed that the occurrence of biofouling communities on cage panels varied significantly among the three depths and between the months. The barnacles and hydroids formed a major contribution of the fouling organisms throughout the study period. The barnacles are the most dominating fouling organisms present on the net panels at 3and 6mt depth. Regular net exchange at monthly intervals and washing and drying of the nets will reduce the fouling effect in marine farm.

5. Acknowledgement

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BIO FOULING ORGANISMS ON THE EXPERIMENTAL PANELS IN CAGE FARM OFF KARWAR, WEST COAST OF INDIA

***Sonali S.Mhaddolkar[1], A.P. Dineshbabu[2], Jayasree Loka[1]**

[1]*Karwar Research Centre of Central Marine Fisheries Research Institute , P.O. Karwar, Uttarkannada, Karnataka, India 581 301*

[2]*Mangalore Research Centre of Central Marine Fisheries Research Institute, PB No 244, Hoige Bazar, Mangalore*

**email: sonamdkar@gmail.com*

ABSTRACT

Biofouling is accumulation of diverse communities of micro as well as macro organisms on submerged surfaces. Present study represents macro and micro biofoulers of marine farm of Karwar, West coast of India during the year 2015-16.The study was carried out for the period of seven months. The net panels were deployed in marine farm at 1 m, 3 m and 6 m depths .This study revealed wide variation in colonization of micro as well as macro organisms between the depths. All together 40, macro and micro biofouling species have been identified from the present study in the cage farm off Karwar, West coast of India.

Key words: Biofouling, micro foulers, macro foulers, cage farm

1.0 INTRODUCTION

Cage farming in open sea is expanding as a major mariculture industry worldwide. In India, successful farming of Asian seabass and cobia in circular GI steel cages was achieved by Central Marine Fisheries Research Institute. Major constraint in cage farming is the biofouling which limits the free flow of water from and into the cages leading to accumulation of organic waste inside the cage. This will eventually cause the outbreak of diseases and mass mortality thereby causing economic loss to the cage farming community. Several reports on the fouling organisms of cage nets and frames were recorded with more intensity in tropical regions (Kassali, 2012; Braithwaite and Mc Evoy, 2004 ; Benson, et al, 1952) which may lead to the disease occurrence with heavy micro fouling organisms (Tan et al., 2002). Though fouling process is dynamic, this can be controlled by the systematic study of major fouling communities, their seasonal dominance and reproductive phases. Hence, a study was undertaken during the period 2015-16 on the occurrence and abundance of micro and macro fouling communities on the net panels near the marine cage farm.

METHOD

The study was carried out during December 2015 - July 2016 in cage farm located at N 14°48.406', E 074°06.664' at Karwar, the Arabian Sea in the west coast of India, (Fig.1 and 2). Experimental panels were made up of half inch PVC pipes with 19 mm dia. and joined with elbow at four corners. The HDPE, net panels of size 100mmx100mm (mesh size 22 mm) were attached using a twine thread. Three square panels were tied on 4 mm nylon rope at three depths and a weight fixed at the other end to stay vertical in the water column. The panels were tied to the floating frames of the cage farm at the selected points and duplicate panels were put at three depths: 1m (top), 3 m (middle) and 6 m (bottom) vertically (fig.3). Microbial samples were collected in the sterile broth media by swabbing 1cm^{2}area from the experimental panel. Other fouling organisms collected were sieved in 200micron sieve and preserved in Rose Bengal and 5% formaldehyde solution. The species were identified (WORMS, 2016) and counted by number of individual organisms per cm^2. Microbial, algal samples analysis and Environmental parameters were done using standard methods (APHA.2005). One way ANOVA analysis was done to understand the variations.

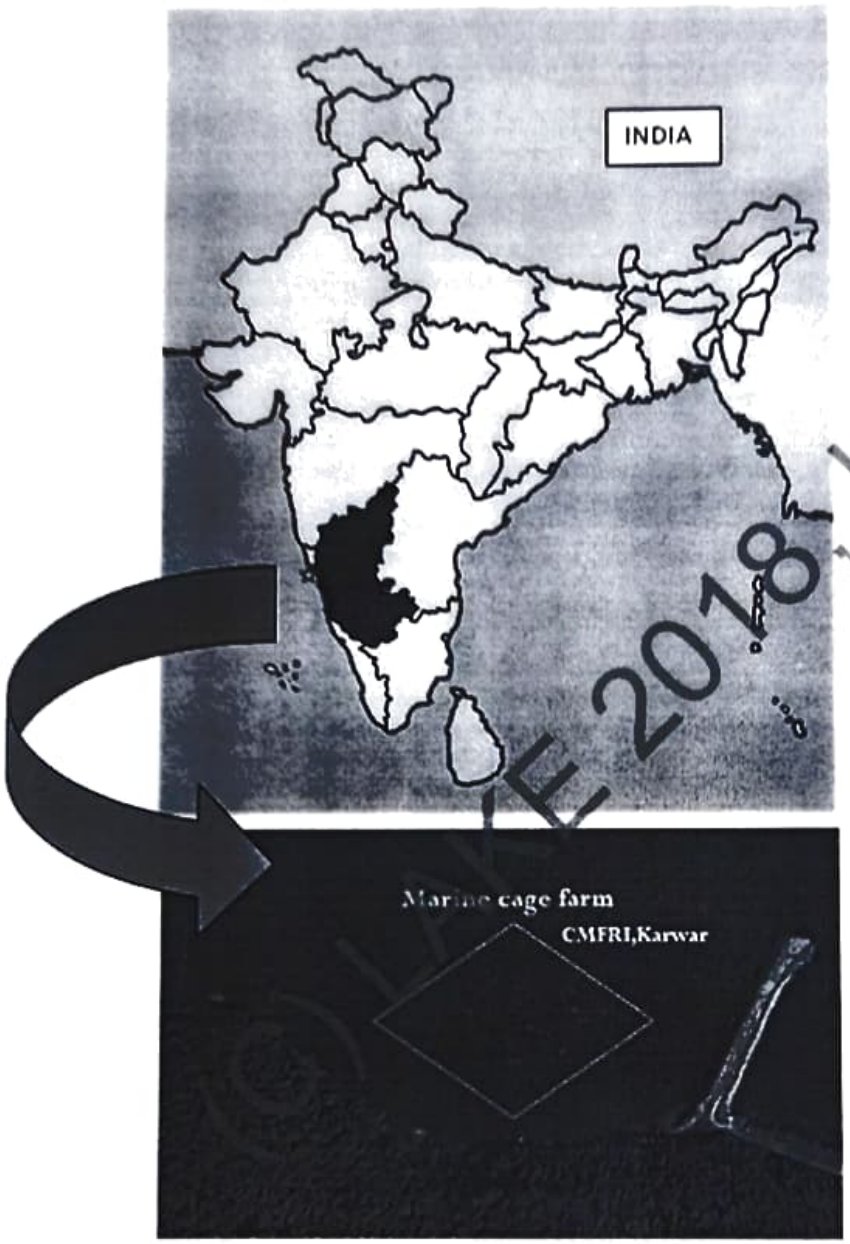

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Fig. 1. the location of the Cage farm of CMFRI, Karwar in west coast of India (Source: Google earth maps, US Dept of state Geographer, Data SIO, NOAA, US, Navy.NGA, GEBCO@2016 Google IMAGE Landsat /Copernicus)

Fig.2. Cage site with experimental panels

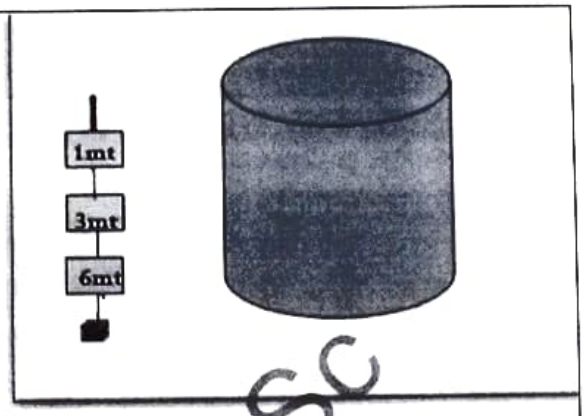

Fig. 3. Represents panel positions at three depths around the cage (yellow rectangular structures represent the panels and blue cylindrical structure represents sea cage).

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3.0 RESULTS AND DISCUSSION

The study revealed wide variation in colonization of micro fouling as well as macro fouling organisms between the depths. Total 40, macro and micro biofouling species have been identified from the experimental net panels in the cage farm off Karwar , West coast of India (table.2).

Microfoulers: Bacteria, diatoms, larval forms are the major microscopic foulers of the net panels. Five bacterial genera, two micro flora and four micro faunal groups were recorded on the experimental panels. Bacteria and diatoms are most bountiful microbes of various fouling surfaces(Prabha devi.1995).The phenomenon of biofilm formation is carried out with a perticular sequence in marine system:conditioning layer is formed, then microbes like bacteria will settle, then EPS (Extracellular-Polymeric-substances) layer is build then the microscopic algae will settle. After that macro foulers will start appearing (Richard et al.2007).

Bacteria: *Vibrio* spp. was found to be the most dominating bacteria on the 3 panels. *Vibrio* loads ranged from to $0.32x10^5$ to $0.93x10^5$ cfu/cm^2 after seven month of installation. Five prominent bacterial genera identified on the submerged net panels, were *Vibrio spp., Aeromonas spp., Alcaligens spp., Alteromonas spp.* and *Pseudomonas spp.* The percentage of *Vibrio* spp. was more on the top and middle panels (71.45% and 71.44%) i.e., similar prevalence between the two panels and comparatively less in the bottom panel (42.86%). *Alteromonas* colonies were found in the middle panels (3m depth). Whereas, the genus *Aeromonas* and *Pseudomonas* were abundant in bottom panels.

Table .1.Bacterial abundance at initial and final fouling on the panels.

TBC(cfu/cm^2)						TVC(cfu/cm^2)					
1 month			7months			1 Month			7months		
1	3	6	1	3	6	1	3	6	1	3	6
$8.35x10^4$	$0.85x10^4$	$49.14x10^4$	$1.8x10^7$	$0.66x10^6$	$0.24x10^6$	$0.46x10^2$	$0.04x10^2$	$0.09x10^2$	$0.32x10^5$	$4.1x10^4$	$0.93x10^5$

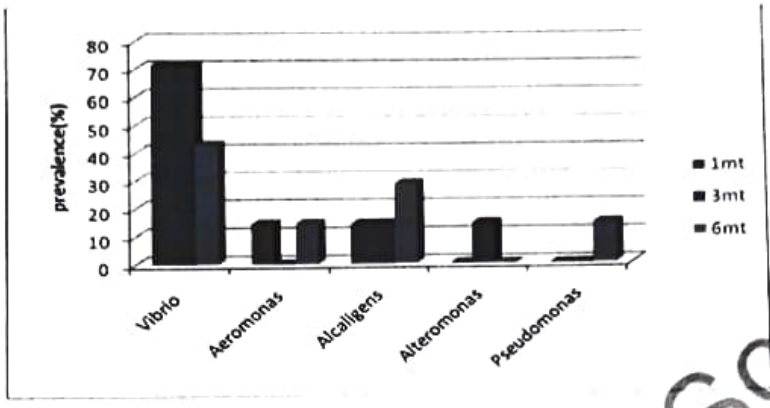

Fig.4. Prevalence (%) of most dominant bacteria isolated from net panel at different depths

Nandhini and Revathi (2016) reported *Bacillus, Vibrio, Pseudomonas, Micrococcus luteus*, *Shigella, Proteus* as the most dominant fouling bacteria isolated on the surface of the boats in Chennai. Vibrio and Pseudomonas were found as abundant indigenous bacterial flora found in most of the coastal fishes (Brown et al. 2000,Sugita et al.1988). Vibrio, Aeromonas, Pseudomonas etc are the opportunistic disease causing bacterial genera to some of the finfishes of aquaculture farms including Sea bass (Vijayan et al. 2015, Seng and Colorni. 2002). Some of the species of vibrios like *V.alginolyticus, V.harveyi* cause mortalities by vibriosis in cage grown fish reported by earlier works in the same site (Sharma *et al.* 2014, Sharma et al. 2016).

Enough references are available for studies on biofilm formation on hard immersed structures like ship hulls and pipes but the literature on the fish culture nets is very scanty (Richard *et al.*) especially in Indian sea cage farming. In present research work prominent bacteria's found attached to the cage net were of Genus Vibrio , Aeromonas , Alcaligens , Alteromonas and Pseudomonas. India there are no much studies on marine attached bacteria's on the cage nets. So the present study will initiate further researches in these aspects.

Microflora: Among the microflora, diatoms and filamentous algae were common. Diatoms were found dominated in all the 3 depths, with a maximum prevalence of 98.04% top panels.

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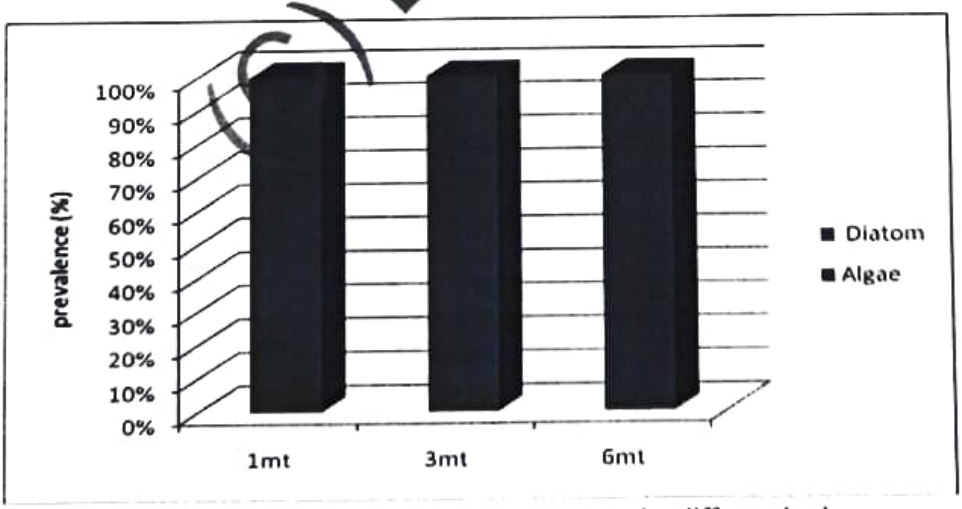

Fig.5. Prevalence (%) of dominant microflora from net panel at different depths.

Among the microflora diatoms were dominant on all the three panels. The 13 diatoms identified were viz. *Cymbella marina, Gymnodinium contortum, Gyrosigma batticum, Licmophora abbreviate, Navicula spp., Pleurosigma normanii, Pseudonischzia spp, Melosira spp ,Nischia spp ,Rhizosolenia spp,. Skeletonema spp., Thalassiosira spp, Thallasionema spp.* Among the diatoms *Navicula* spp. were dominant 85% with density of $1.2x10^5$cells/cm^2 on the top panels.

High density and diverse species ranges of diatoms are present in marine biofilm (Cooksey et al.1984). Navicula, Nitzchia, Licmophora, Amphora, Cylindrotheaca are dominant marine micro fouling diatoms (Patil and Anil, 2005) on various marine submerged surfaces. Due to the presence of raphe the pinnate diatoms attach on the substratum (Molina and Wehebee.2008). Present study revealed the similar results on the experimental net panels with *Navicula* sp. as a dominant diatom in the micro fouling community other than bacteria.

Many Studies have been revealed that Centric diatoms are bountiful in water column but pinnate diatoms dominate in fouling communities of different surfaces in estuarine and marine waters (Prabha devi.1995,Characklis and Cooksey. 1983), the results are true for the cage net panel studies in the present case. Seven month panels showed the abundance of pinnate diatoms. *Navicula* spp. were dominant ($1.2x10^5$cells/cm^2) on the top panels.In present research the prevalence of diatom is more in 1 meter depth net panels when compared to the other panels which may be due to the light penetration.

Microfauna: Among the fouling microfauna four groups were common in all the three depths. They are worms, larval forms, copepods, protozoans. Among them larval forms were most dominant (67%) at 3 mt, Protozoans were least dominant (1%) at 1mt and 3mt panels. Also copepods were least dominant (1%) at 6mt depth panels.

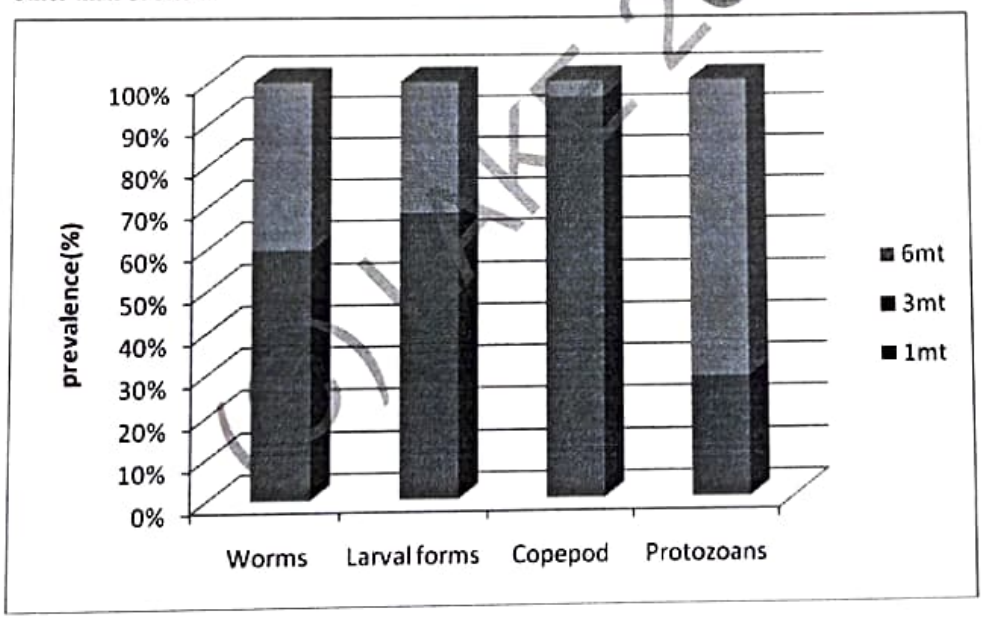

Fig.6. Prevalence (%) of micro fauna from net panel at different depths.

Overall four micro faunal groups were identified on the panels. Among the worms Polychaete worms (40%) and Nematode worm (60%) were common. Among the copepods herpacticoid copepods were common. Among the larval forms Lamellibranch larvae (90%) and Nereid larva (10%) were prevalent. Among the protozoans, Foraminiferans were prevalent. Among all, Lamellibranch larvae were most abundant micro fauna of the middle panels after seven month of immersion.

Most of the larvae of bentic and fouling organisms get carried to the fresh settlement sites by water current (Pudota. J. 2011) then settle and form the macro fouling

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communities. The settlement mainly depends on sunken material surface (Larrson, 1997) and presence of biofouler larvae in surrounding water. Green mussel larvae have been recorded on the net panels by Pudota. J. 2011, In the present research work the lamellibranches larvae were found dominant among the fouling micro fauna, lamellibranches larvae included both Modiolus and green mussel.

Macrofoulers: Total of 22 species of macrofouling organisms were identified from the marine cage farm during this study. *Chondrilla spp. , Obelia spp ,Oliva gibbosa, Oliva inspidula, Perna viridis, Modiolus spp., Crassostrea spp., Saccostrea cucculata, Platynereies spp., Leptoplana spp, Hyperia spp., Caprellid spp., Amphibalanus spp., Grapsus albolineatus, Schizophrys aspera, Ozius rugulosus ,Medaeus granulosus , Plagusia squamosa, Idotea spp., Bunudosoma spp., Stomopneustes variolaris, Ascidia spp.* Hydroids and barnacles were prominent throughout the culture period.

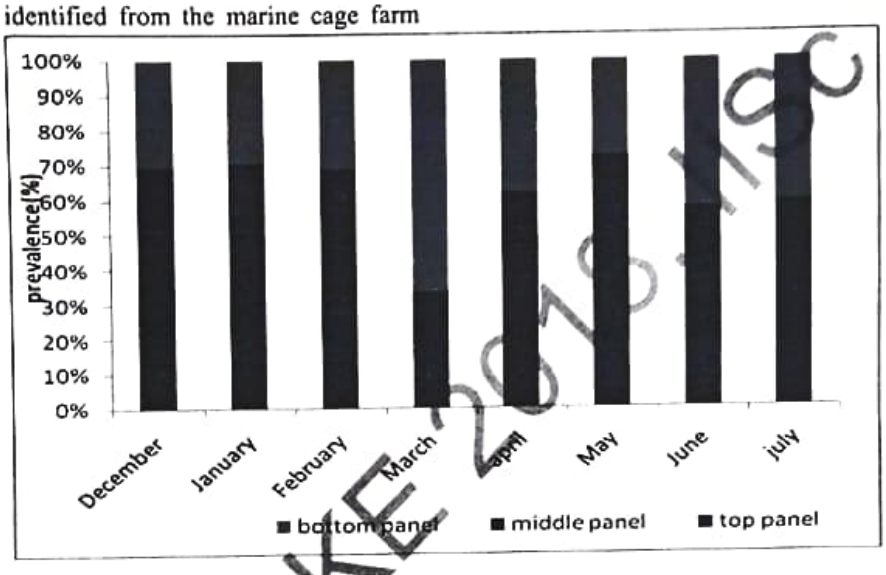

Fig.7. Prevalence (%) of Barnacles from net panel at different depths.

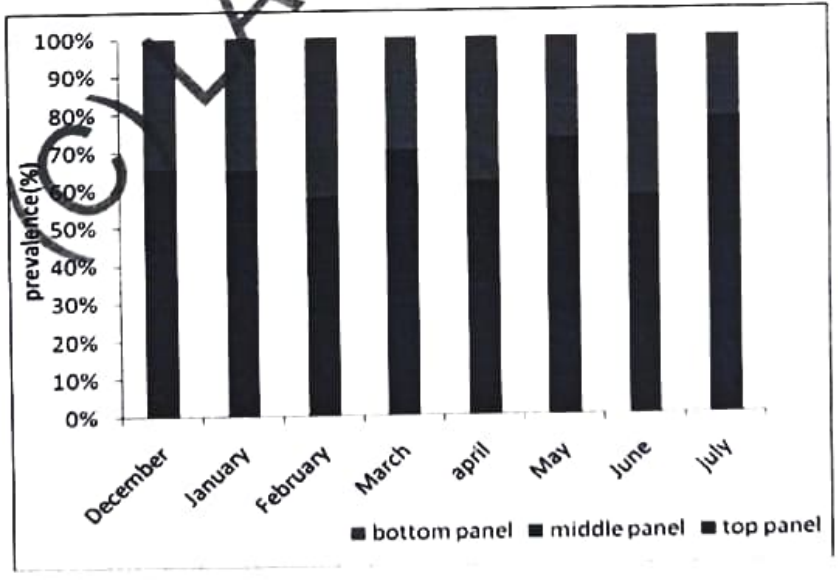

Fig.8. Prevalence(%) of Hydroids from net panel at different depths.

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Sevel workers reported that there is significant variation of assemblege of foulers of net with depth (Kassah, 2012, Svane, Cheshing and Barnett, 2006, Nellis and Bourget ,1996).In the prasent study Barnacles and Hydroids were most common macro foulers through out the study period.Occurance of Hydroids also has significant variation with depth in culture cage-nets(Kassah, 2012, Nellis and Bourget,1996, Guenther et al., 2010).In the prasent work also variation is recorded in accordance with depth. Hydroids were most dominant in the top panel with 91.67% dominace in July and least dominant during February (1.8%) at the bottom panel.

Balanus amphitrite was considerable fouler reported in the pearl oyster culture farm in the Gulf of Mannar. Dharmaraj, and Chellam,1982 studied panels in the depth ranges from 0.5m to 2meter and observed a heavy settlement during June. As in the present case barnacles were common foulers throughout the culture period. Barnacles were dominant during May and March (93.77% and 93.73%) in the middle and bottom panel and least dominant during March (7.14%) in the top panel. A significant variation was recorded in the occurrence of barnacles and hydroids between the three depths ($p<0.05$).

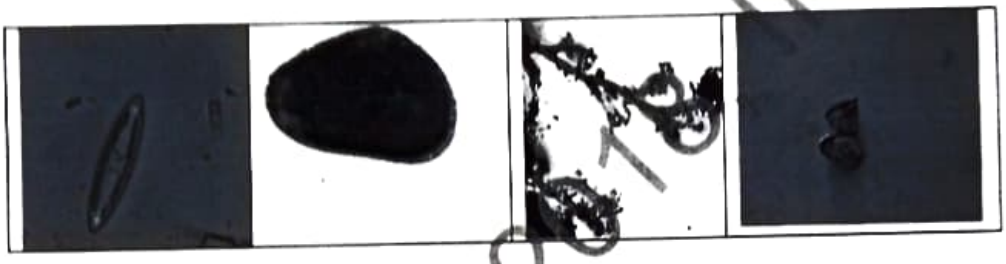

Fig.9. Micro and macro foulers of net panels: *Navicula*, Lamellibranch larvae, Hydroids, Barnacles.

Table. 2. biofouling organisms identified on the experimental panels in the cage farm off Karwar, West coast of India

Sl.No	Species identified	Biofouling group
1	*Vibrio spp.*	
2	*Aeromonas spp.*	
3	*Alcaligens spp.*	
4	*Alteromonas spp.*	
5	*Pseudomonas spp*	Bacteria
6	*Cymbella marina*	
7	*Gymnodinium contortum*	
8	*Gyrosigma hatticum*	
9	*Licmophora abbreviate*	
10	*Navicula spp.*	
11	*Pleurosigma normanii,*	
12	*Pseudonischzia spp.*	
13	*Melosira spp.*	
14	*Nischia spp.*	
15	*Rhizosolenia spp.*	
16	*Skeletonema spp.*	
17	*Thalassiosira spp.*	Diatom

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18	*Thallasionema spp.*	
19	*Chondrilla spp.*	Sponge
20	*Obelia spp.*	Hydroids
21	*Oliva inspidula*	Gastropod
22	*Oliva gibbosa*	
23	*Perna viridis*	Green mussel
24	*Modiolus spp.*	Modiolus
25	*Crassostrea madrasensis*	
26	*Saccostrea cucullata*	Oyster
27	*Platynereies spp.*	Polycheate worms
28	*Leptoplana spp.*	Flat worm
29	*Hyperia spp.*	
30	*Caprellid spp.*	Amphipod
31	*Amphibalanus spp.*	Barnacles
32	*Grapsus albolineatus*	
33	*Schizophrys aspera*	
34	*Ozius rugulosus*	
35	*Medaeus granulosus*	
36	*Plagusia squamosa*	Crab
37	*Idotea spp.*	Isopods
38	*Bunudosoma sp.*	Sea anemone
39	*Stomopneustes variolaris*	Sea urchin
40	*Ascidia spp.*	Ascidia

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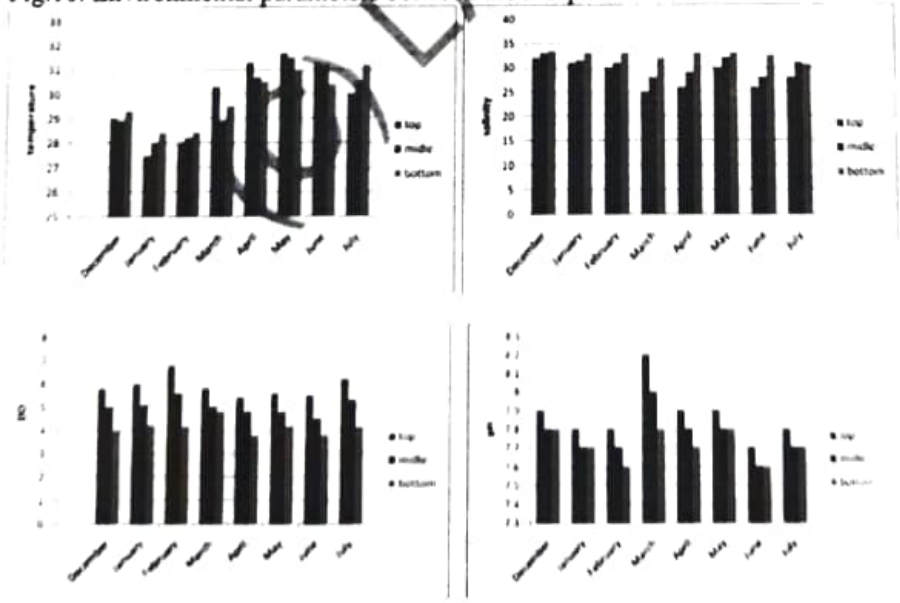
Fig.10. Environmental parameters between the 3 depths



The environmental parameters varied between the three depths during the study period. Environmental parameters such as Salinity, Temperature, pH, Dissolved Oxygen appeared to have significant contributions on biofouling settlements. A significant impact of water quality of cage site in the settlements of fouling organisms was recorded (Fig.10). The study revealed a significant variation in colonization of micro as well as macro organisms between the depths. Among microfouling organisms, bacteria (*Vibrio* spp.), diatoms (*Navicula spp.*) and Lamellibranch larvae were found dominated on top and middle panels after 7 months of experiment. Among the macrofoulers, Barnacles dominated in the middle (3mt) and bottom (6mt) panel, where as hydroids were dominant on top (1mt) panels.

Impact of bacterial and other microfoulers dominance on the occurrence and abundance of macrofouling organisms on the net panels at different depths is recorded during this study. A direct correlation between the temperature and *Vibrio* abundance was recorded during the study. It was also recorded that there is a direct impact on the abundance of *Vibrio* loads on the fouling of barnacles on net panels in all the three depths. Hence, it can be stated that temperature and microfauna plays a significant role on the occurrence and abundance of marofoulers on panels. Further studies are needed to understand the interrelation between the temperature and occurrence of Vibrios and other fouling organisms. However, net exchange at monthly intervals is recommended to eliminate and prevent the biofouling on the cage nets.

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Theme IV

MICRO AND MACRO FOULING COMMUNITIES ON THE EXPERIMENTAL NET PANELS IN MARINE CAGE FARM OF KARWAR, WEST COAST OF INDIA

Sonali S. Mhaddolkar, Jayasree Loka, Philipose K.K. and Dineshbabu A.P.
Mangalore Research centre of Central Marine fisheries Research Institute, Mangalore

A study was carried out at the marine farm of Karwar, to assess the colonization, fouling pattern and species dominance of micro and macro biofouling organisms in cage culture site of West coast of India during the year 2014-15.The study was carried out for the period of seven months. The net panels of mesh size 22mm were deployed in cage site at 3 different depths (1, 3and 6 meters) and the study revealed wide variation in colonization of micro as well as macro organisms between the depths. Bacteria, diatoms, Larval forms are the major microscopic foulers of the net panels. The *Vibrio* spp. are the dominating bacteria on the 3 panels. Vibrio loads ranged from to 0.32x105 to 0.93x105 cfu/cm^2 after 30 weeks of installation. Five prominent bacterial genera identified on the submerged net panels, were *Vibrio* spp., Aeromonas spp, Alcligens spp., Alteromonas spp. and Pseudomonas spp. The percentage of Vibrio spp. was more in the top and middle panels (71%) and comparatively less in the bottom panel (42.86%). Among the diatoms Navicula spp. were dominant (1.2x105cells/cm2) on the top panels. Among the micro fauna, Lamellibranch larvae were abundant on the panels after 30 weeks of fouling. The density of macrofoulers was compared between the three depths, and it was found that density is more in bottom panels than top and middle panels. Hydroids were prominent throughout the culture period, whereas Barnacles were the most dominant (93.75%) fouling communities among the macro foulers, on the net panels. A significant impact of water quality of cage site in the settlements of fouling organisms was recorded.

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This is to certify that Dr/Sri/Smt. Sonali S. Thandolkar has participated/ presented paper in the National Workshop on Marine Biodiversity & Climate Change organized by the Central Marine Fisheries Research Institute, Karwar Research Centre, Karwar, Uttara Kannada, Karnataka on 21st and 22nd May 2014.

Dr. A Gopalakrishnan,
Director, CMFRI

Dr.K.K. Philipose
Organizing Secretary

BIODIVERSITY STUDIES IN THE MARINE CAGE FARM OFF KARWAR

Sonali Mhaddolkar, N.G.Vaidya, Jayasree Loka, Krupesha Sharma and K.K.Philipose

Karwar Research Centre of CMFRI, P.O. Karwar, Uttar Kannada, 581301

Open sea cage culture of marine fin fishes and shell fishes has been the major activity of Karwar Research Centre of CMFRI. The marine farm of the centre holds 25 numbers of cages of varying sizes in which seven species of finfish and mussel is being farmed since 2009. The coastal waters off Karwar are highly dynamic and hold a variety of diversified finfish and shellfish fauna which have impact over cage culture systems. Hence, a study has been carried out to understand and assess the marine biodiversity in the cage farm of Karwar during the period 2009-2014. The study revealed that *Ambassis* spp. was the most dominant species throughout the culture period during all the years. Among shellfish, Modiolus sp. was observed as the most dominant species recorded in all the seasons

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This certificate is awarded to Ms. Sonali S. Mhaddolkar From Central Marine Fisheries Research Institute for her poster presentation of research paper titled Environmental influences on the biofouling biomass in open sea cage culture site in the "3rd National Seminar on Current Trends in Life Sciences" held on January 29, 2021.

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PP-45

Environmental influences on the biofouling biomass in open sea cage culture site

Sonali S.Mhaddolkar[1] and A.P.Dineshbabu[2]

1, Mangalore University, Karnataka & CMFRI Karwar Research Centre, Karwar, Uttarkannada, 581301

2, Mangalore Research Centre of CMFRI, PB No.244, Hoige Bazar, Mangalore

*Email:sonamdkar@gmail.com

Introduction

In the cage culture practise biofouling is a major and continuous problem for the fish farmers (Pudota,2011, Hodson et. al 1997). Worldwide many studies have been conducted on the biofoulers aggregation on the salmon culture infrastructure facilities (Fitridge et. al. 2012). Globally, sea cage farming population is busy in finding out cost effective methods to solve the constrains of cage culture like labour requirement, biofouling problem, environmental issues etc. and it varies with the location and environment(Pudota,2011; Braithwaite and Mc Evoy 2005). Before implementing the new methods in this it is very much necessary to have the complete knowledge of culture site and to investigate the biological aspects like biofouling and their relationship with the environmental parameters. Biofouling intensity, biomass, density, species composition and the correlation with environment are the important aspects to be considered in this field to overcome the net blockages, high drag, diseases related to biofouling in that locale and making the cage culture profitable and economical to the farmers.

The present research is to assess the biomass of foulers in the experimental net panels deployed in varied environmental features, cage culture site and the reference site to understand the influence of the environment on biofouler accumulation.

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BIO FOULING ORGANISMS ON THE EXPERIMENTAL PANELS IN CAGE FARM OFF KARWAR, WEST COAST OF INDIA

***Sonali S.Mhaddolkar[1], A.P. Dineshbabu[2], Jayasree Loka[1]**

[1]Karwar Research Centre of Central Marine Fisheries Research Institute , P.O. Karwar, Uttarkannada, Karnataka, India 581 301

[2]Mangalore Research Centre of Central Marine Fisheries Research Institute, PB No.244, Hoige Bazar, Mangalore
**email: sonamdkar@gmail.com*

ABSTRACT

Biofouling is accumulation of diverse communities of micro as well as macro organisms on submerged surfaces. Present study represents macro and micro biofoulers of marine farm of Karwar, West coast of India during the year 2015-16.The study was carried out for the period of seven months. The net panels were deployed in marine farm at 1 m, 3 m and 6 m depths .This study revealed wide variation in colonization of micro as well as macro organisms between the depths. All together 40, macro and micro biofouling species have been identified from the present study in the cage farm off Karwar, West coast of India.

Key words: Biofouling, micro foulers, macro foulers, cage farm